New Series Vol. XIX No. 76 October 1955

Price 20*s*. net

ANNALS OF BOTANY

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OXFORD : AT THE CLARENDON PRESS
LONDON : GEOFFREY CUMBERLEGE

PRINTED IN GREAT BRITAIN
BY CHARLES BATEY AT THE UNIVERSITY PRESS, OXFORD

1955

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The Rate of Transport of Natural Auxin in Woody Shoots

BY

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AND

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With five Figures in the Text

Abstract

A method is described for the estimation of the rate of movement and the quantity transported of the natural growth hormone in standard isolated segments of apple shoots. During controlled storage diffusible auxin is collected, and later by dividing the standard length of stem into small sections the location of the auxin front is determined, from which the rate of transport is deduced. Temperature markedly affects both rate of transport and amount of auxin transported (cf. van der Weij, 1932), a maximum occurring at 27–30° C., followed by a rapid fall to zero. The total diffusible auxin in a given length of stem is not affected by storage temperatures below 30° C. but falls to zero at 42° C. The rate of transport and amount transported are proportional to the oxygen tension over the range 0 to 5 per cent. O_2, and there is some evidence for destruction of auxin in tensions below 2 per cent.

Introduction

SINCE the first collection of auxin from *Avena* coleoptiles by Went (1926) determinations of free moving auxin have been made on leaves, stems, and terminal buds of both herbaceous and woody plants. The procedure adopted has been to mount the basal cut surface of the plant organ on small agar plates into which the mobile auxin is allowed to diffuse, followed by a quantitative assay using the *Avena* curvature test. This collection method depends upon the polar transport of auxin demonstrated by Went (1928, 1939) in the *Avena* coleoptile and shown to occur even against a concentration gradient (van der Weij, 1932). Though this polar transport has now been confirmed in a very wide range of both herbaceous and woody species (Avery et al., 1937; Clark, 1937; Went and Thimann, 1937, p. 97; Oserkowsky, 1942; Jacobs, 1950, 1952), the rate at which this transport occurs has so far only been examined directly in the *Avena* coleoptile by van der Weij (1932) and Went and White (1939), and in the gynophore of *Arachis hypogaea* by Jacobs (1951).

The technique of van der Weij and Jacobs depended on estimates of the amounts of auxin which passed during various intervals of time from an auxin source applied at the upper end of a cylinder of tissue to a plain agar plate at the lower end. Extrapolation of the curves so obtained gave an estimate of the time at which auxin first arrived at the base of the cylinder, and hence

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]

afforded an estimate of the transport rate. Went and White placed cylinders of plant tissue, carrying at the upper end an agar block containing auxin, directly on the coleoptile, and used the photokymograph to determine the time after which curvatures began. By deducting the time required for curvatures to occur in coleoptiles omitting the transporting cylinder of tissue, an estimate of the transport rate through the material was deduced.

From the early work of Dolk (1929, 1936) in which phototropic, geotropic, and applied auxin curvatures are compared, an estimated rate of auxin transport of about 1·0 to 1·5 cm./hr. has been determined (Du Buy and Nuernbergk, 1932). Thimann (1934) deduced the rate of transport in buds of *Vicia*. The growth substance obtained from the buds by extraction approximately equalled the amount which would diffuse into agar in 1 hour (Thimann and Skoog, 1933), and it was concluded that the growth substance required 1 hour to travel from the extreme tip of the bud to the cut surface about 1 cm. distant and indicated a rate of movement of about 1 cm./hr., thus agreeing reasonably with other estimates of transport rate. Skoog (1938) examined sections at various positions down the stems of squash plants several hours after applying physiological concentrations of growth substance in lanolin, and from the data it would appear that the auxin had moved about 10 cm. in 5–6 hours.

van der Weij (1932) presented a number of graphs showing the effect of temperature on the rate of auxin transport through sections of *Avena* coleoptile. To the data obtained were fitted straight lines which when extrapolated met at a single point on the abscissa, from which result he made the important claim that temperature affected the quantity of auxin transported but was without effect on the velocity of transport. Du Buy and Nuernbergk (1932) and also Boysen Jensen (1936) have pointed out that the lines fitted were somewhat arbitrary, and this point will be dealt with later in this paper.

The work described here has been undertaken to study the transport of auxin in the woody shoots of the apple, and in particular to examine again the effect of temperature and oxygen concentration upon the movement both as regards quantity and rate of transport. A new and very simple technique is used which has the advantage that the rate of movement of growth substance in the plant is measured directly, making use of the naturally occurring auxin of the tissue.

Using the diffusion technique Hatcher (1948) collected auxin from isolated segments of apple shoots. When collection from single 1-cm. lengths was made in successive hourly periods the amount of auxin accumulated in the agar plate during the second hour was considerably greater than that in the first. This, he supposed, was due to the fact that auxin was arriving at the base of the section faster than it could diffuse away in the agar and so the concentration rose until the gradient from tissue to agar was steep enough to ensure equality of transport and diffusion rates. This suggestion was confirmed by keeping the stem segment in a humid atmosphere, which resulted in a high concentration of auxin at the lower cut surface of the tissue, and there was now no difference in the amount of auxin collected in an agar block

in the first and second hours. When the shoot segments which had been stored in this way for 2 hours were divided into four sections and each separately assayed, the two uppermost were found to be entirely devoid of auxin, while only the extreme basal section gave the characteristic rapid delivery of auxin in the first hour. In a further experiment, using a 10-cm. length of stem, it was found that the uppermost 1 cm. was clear of auxin after 2 hours. These data provided the first estimates of rate of transport of auxin in woody shoots (Hatcher, unpub.).

Preliminary Estimates of Transport Rate of Natural Auxin

Materials and methods

Preliminary experiments were carried out in August and September 1949, using stoolbed shoots of Crab C apple. At the beginning of this period the shoots were about 95 cm. long with 35 internodes and free-moving auxin

Table I

Distribution of Auxin in Stem Segments of Varying Length after 2 Hours' Storage. (*Degrees curvature*)

Length of segment.	1-cm. sections from apex to base.				
	1.	2.	3.	4.	5.
1 cm.	35·0				
2 cm.	0	31·8			
3 cm.	0	21·3	35·3		
5 cm.	0	23·5	25·1	23·0	33·0

could be detected over the whole length by the diffusion technique (Hatcher, 1948), but a much higher content was present in the upper part of the shoot in the region of the fifth internode; this region of the shoot was therefore used in all the following experiments.

Shoots freshly collected were used for each test, the required portion of the shoot was isolated, and all leaves together with their axillary buds removed immediately before the experiment was begun. The stem segment was stored in the dark at a high humidity for a known period and then subdivided as required; the auxin content of the individual sections was assessed by collection into agar plates and assayed by the modified *Avena* curvature technique (Rawes and Hatcher, 1949).

Transport of natural auxin in isolated stem segments of various lengths

Table I shows the distribution of auxin in stem segments of length 1, 2, 3, and 5 cm. after storage for 2 hours in a humid atmosphere. Stem segments which had not been stored showed a fairly uniform distribution of auxin throughout, while after storage it is seen that the first centimetre contained no auxin. The transport rate was thus 0·5 cm./hr.

In a similar experiment in which four stem lengths each of 30 cm. were stored for 18 hours, three showed 10 cm. and one 12 cm. of the stem clear of auxin. The rate of transport thus varied from 0·55 to 0·66 cm./hr. These experiments indicated that the rate of transport is independent of the length of the stem segment used (as found by van der Weij for the coleoptile) and also of the period during which transport continues. The latter point was further confirmed in an experiment in which 30-cm. segments were stored for 4, 6, 8, and 18 hours, and showed respectively 2, 4, 5, and 13 cm. of the tissues evacuated; this represents transport rates of 0·50, 0·67, 0·63, and 0·72 cm./hr.

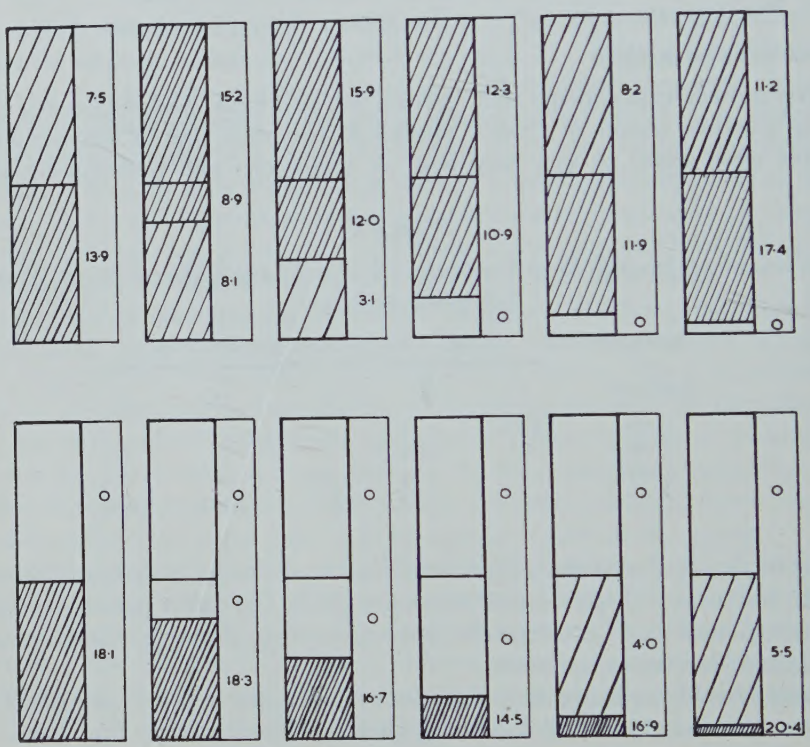

FIG. 1. Distribution of auxin in 2-cm. segments of Crab C shoot. The upper row are unstored segments, the lower row segments which have had 2 hours' storage. On the extreme left the segments have been divided into two 1-cm. sections, in the remainder the lower centimetre has been divided in the following ratios, 1:3, 1:1, 3:1, 7:1, 15:1. The figures show the degrees curvature given by the individual sections when diffused into 0·04 ml. agar. The shading indicates the concentrations of auxin in the sections.

Site of auxin accumulation during storage

In the experiments described above it was observed that a high concentration of auxin occurred in the basal centimetre of the stored shoots, and the two following experiments were designed to ascertain how localized this region of high concentration was. Two-centimetre segments were stored for 2 hours and then examined in three portions; in all cases the top section was

1 cm. long, while the lower centimetre was divided in the following ratios, 1:3, 1:1, 3:1, 7:1, 15:1. Similar segments were examined without any storage period. Diffusion from the fractional portions was carried out for 1 hour into agar plates 0·04 ml. in volume.

The distribution of auxin in the different regions of the stem segment before and after storage is shown graphically in Fig. 1. It will be seen that before storage the auxin is more or less uniformly distributed throughout the segment, but after 2 hours has moved to the base and has become concentrated in the basal $\frac{1}{4}$ cm.; indeed the major part is present in the basal $\frac{1}{16}$ cm. Though this concentration occurs in the extreme base of the section it has been shown to be retained within the tissue and is not free on the surface; for in a further experiment the basal surface of the lower section was either scraped with a sharp blade to remove the surface cells or was washed for about 2 minutes with a strong jet of water, and yet the amount of auxin collected was only slightly less than in untreated sections.

Influence of Temperature upon Rate of Auxin Transport

Material and method

These experiments were carried out during the next season's growth of the stoolbed shoots of Crab C, from June 1950, when they were about 50 cm. long, until mid-September, when they were about 130 cm. long. The portion of shoot used, however, was always the same relative to the apex, consisting of the 5 cm. of stem taken from just below the 4th expanded leaf, which as previously stated is the region with the maximum amount of auxin per unit length of shoot.

Throughout the series of experiments these 5-cm. lengths of tissue were cut from freshly collected shoots. After removal of the leaves and axillary buds an initial storage period was given at various temperatures during which auxin was allowed to diffuse out into agar applied to the lower cut surface.

At the end of the storage period the stem segment was removed and cut into $\frac{1}{2}$-cm. sections (except in expt. 167, where 1-cm. sections were used). The sections were placed in serial order on microscope slides with their basal ends in contact with agar plates, which were of 0·09 ml. volume when the section contained large amounts of auxin, but were reduced to 0·04 ml. for expts. 208 and 212 which were carried out late in the season when the total auxin of the shoots had fallen. After 1 hour the agar plates were changed and collection continued for a further 2 hours. Experience showed that after this time no further auxin could be obtained by again replacing the agar plates. Diffusions were carried out in darkened dishes which were maintained at a high humidity with water-saturated 'clarity sheet' (Keyworth, 1951).

The agar plates together with those used for the collection during the original storage period were assayed for auxin by the modified *Avena* curvature technique previously mentioned. A group of coleoptiles received either the nine unit blocks (0·01 ml. each) cut from a single agar plate, or where

plates of 0·04 ml. were used, blocks from two plates of the same treatment. The blocks obtained from the consecutive ½-cm. sections from each shoot were tested and the number of sections which had been evacuated of auxin during the storage period was determined by the number of sets of coleoptiles in which no curvature occurred. From this the rate at which the auxin moved in the stem segment can be determined. No measurement of actual degree of curvature is necessary to determine the *rate* of transport, though this is necessary for estimating the *amounts* of auxin transported as well as the total amount of auxin present in the original stem segment. This elimination of the need to measure curvatures is a great advantage since it means that transport rates can be derived even when the curvatures obtained lie outside the proportionality range, and also it circumvents the errors resulting from variation in sensitivity during the course of a test.

In order to obtain a precise estimate of the rate of movement of the auxin in the stem segment, the period of storage should be long enough to ensure at least 50 per cent. of the original 5 cm. depleted, and this should be followed by examination in small sections. The number of agar plates which could be examined in a curvature test limited the number of sections to ten, each of ½ cm. To estimate the amounts of auxin transported and the changes in total auxin content in the stem segment it is necessary to ensure that the volume of agar used for collection is such that the concentration is not high enough to overstep the proportionality range in the curvature test. Attempts were made to achieve this in the earlier experiments by frequently changing the agar plates, but in the later experiments a more satisfactory method was devised in which collection was made into 0·5 ml. of agar for 4 hours at temperatures below 17° C. and for 3 hours for temperatures of 17° C. and above. The agar was later melted down and part cast into a plate of 0·12 ml. (12 unit blocks). The remainder of the agar was kept in reserve in a refrigerator and could be further diluted should the curvatures obtained in the test exceed the proportionality range.

Because of the time taken to carry out such transport experiments it was not possible to perform the *Avena* curvature test on the day of collection of auxin, and the blocks were therefore placed on microscope slides in a closed shallow dish, the atmosphere of which was maintained at a high humidity, and kept at a temperature of about 3° C. in a refrigerator. In a number of preliminary experiments it was shown that blocks stored in this way for 24 hours lost none of their activity.

Temperature control

Constant temperature conditions during storage of the segments were maintained by the use of 1-litre wide-necked cylindrical vacuum flasks, which were provided with a bung to which were sealed three corks fitting into specimen tubes 3 in. × 1 in. These larger tubes held a few millilitres of water to maintain saturation; and into them were placed smaller open tubes containing the stem segments under investigation. The larger tubes dipped into

water at the required temperature. After 1 hour to reach thermal equilibrium the segments were rapidly introduced, and at intervals the temperature of the water was checked. By this simple technique temperatures well above and

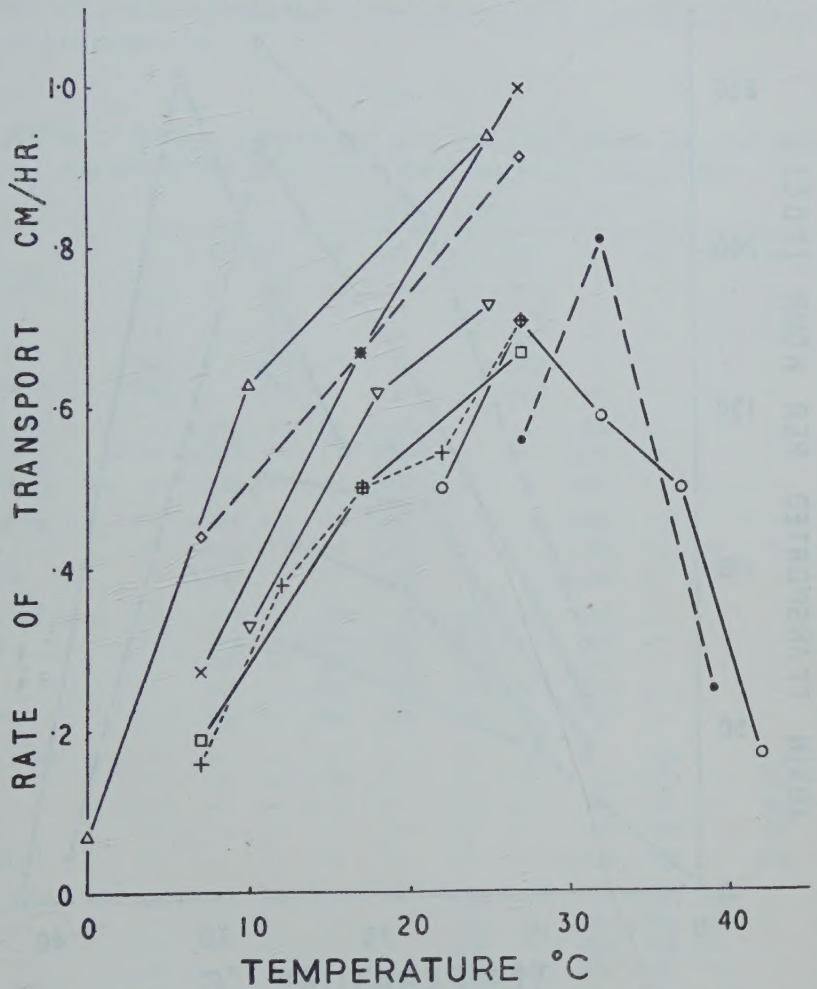

FIG. 2. The effect of temperature on the rate of transport of auxin. The symbols used for each experiment are the same as those used in Fig. 3.

below room temperature could easily be maintained and only rarely was it necessary to adjust the temperature by the addition of ice or hot water.

At the end of the storage period collection of auxin from the sections into which the stem segment was cut was always carried out in an incubator at 25° C.

Results

The results of eight experiments are shown graphically in Figs. 2 and 3 covering a range of temperatures from 0° C.–42° C.

In Fig. 2 the rate of transport in cm./hr. is shown. This is almost zero at 0° C., increases to an optimum rate around 27° C., and declines rapidly above this temperature. The Q_{10} between 10° C. and 30° C. is of the order of 2.

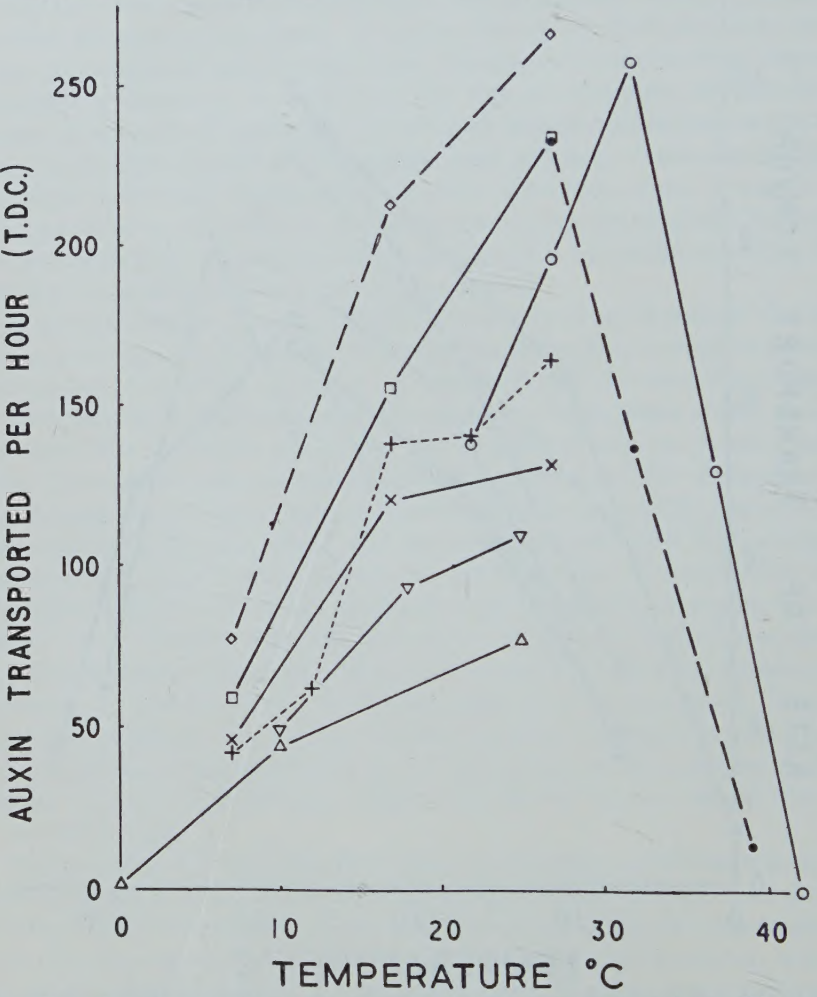

FIG. 3. The effect of temperature on the amount of auxin transported per hour. The symbols used for each experiment are the same as those used in Fig. 2.

In Fig. 3 the total amount of auxin transported out of the stem per hour during the storage period is shown, the values being the total degrees curvature, i.e. the product of the mean curvature per unit block used in the test and the number of such units contained in the original volume of agar used for collection. Contrary to the views expressed by van der Weij, the total amount of auxin transported closely follows the changes in rate of transport, showing an optimum value at between 27° C. and 32° C. The flattening of some of the curves near the optimum is due to the concentration of the auxin

collected being beyond the proportionality range of the curvature test. Above 32° C. the amount of auxin transported rapidly falls off, reaching zero at 42° C.

From the experimental data a further important estimate can be made, namely, the possible production or disappearance of auxin during storage at different temperatures.

TABLE II

The Effect of Storage Temperature upon the Total Auxin (in total degrees curvature) obtainable from a 5-cm. Length of Crab C Shoot

	Storage temperature °C.											
	0.	7.	10.	12.	17.	22.	25.	27.	32.	37.	39.	42.
Expt. 167	491	—	550	—	—	—	508	—	—	—	—	—
	413	—	703	—	—	—	398	—	—	—	—	—
	—	—	443	—	—	—	437	—	—	—	—	—
	275	—	367	—	—	—	554	—	—	—	—	—
Mean	**393**	—	**516**	—	—	—	**475**	—	—	—	—	—
Expt. 181	—	1,519	—	—	1,290	—	—	1,686	—	—	—	—
	—	1,866	—	—	1,894	—	—	1,794	—	—	—	—
Mean	—	**1,693**	—	—	**1,592**	—	—	**1,740**	—	—	—	—
Expt. 184	—	1,064	—	—	2,196	—	—	1,680	—	—	—	—
	—	1,415	—	—	1,768	—	—	962	—	—	—	—
Mean	—	**1,240**	—	—	**1,982**	—	—	**1,321**	—	—	—	—
Expt. 208	—	710	—	706	740	1,055	—	785	—	—	—	—
	—	859	—	761	1,083	827	—	916	—	—	—	—
	—	826	—	568	999	862	—	903	—	—	—	—
	—	984	—	693	807	852	—	820	—	—	—	—
Mean	—	**845**	—	**682**	**907**	**899**	—	**856**	—	—	—	—
Expt. 202	—	—	—	—	—	—	—	—	787	—	574	—
	—	—	—	—	—	—	—	1,444	782	—	646	—
	—	—	—	—	—	—	—	721	667	—	504	—
	—	—	—	—	—	—	—	932	860	—	350	—
Mean	—	—	—	—	—	—	—	**1,032**	**774**	—	**519**	—
Expt. 212	—	—	—	—	—	711	—	..	1,193	562	—	262
	—	—	—	—	—	548	—	1,202	1,899	984	—	213
	—	—	—	—	—	1,176	—	838	848	747	—	150
	—	—	—	—	—	828	—	906	823	619	—	458
Mean	—	—	—	—	—	**816**	—	**982**	**1,191**	**728**	—	**271**

Total auxin in the stem segments was obtained by adding together the auxin collected during the 3 hours' storage and that obtained by diffusion from the $\frac{1}{2}$-cm. sections into which the stem was later cut. The values as total degrees curvature are entered in Table II.

Although there is considerable variability between replicates and treatments, it is probable that there is no differential effect of temperature on total auxin content up to 32° C., nor so far as the evidence goes is there any marked production of auxin at the optimal temperature for transport. Statistical analysis showed no significant differences in total auxin in the experiments at

temperatures of 32° C. or below; on the other hand, above 32° C. the rapid decline in total auxin had high statistical significance and either destruction, inactivation, or inhibitor production must have occurred.

Influence of Oxygen Concentration on Rate of Movement

Method

Shoots of Crab C were again used in these experiments which were carried out from June to September 1951. The conditions of storage were similar to those used in the temperature experiments already described. Five-centimetre segments were stored for 3 hours with their basal ends in contact with 0·5 ml. agar and were maintained during this period at 25° C. Known mixtures of nitrogen and oxygen were passed over these stem segments in the following manner during storage. An aspirator jar was filled with the mixture of nitrogen and oxygen by displacing known volumes of water by each of the gases in turn at atmospheric pressure, from cylinders of the pure gas. By running water into the aspirator the gas mixture was displaced and passed over the sections. The tubes were swept out with a rapid flow of the gas mixture as soon as the stem segments were in place and later a slow constant stream of gas was maintained. The gas mixture passed first through a small volume of water, which served both to humidify it before it passed over the sections and to indicate the rate of flow. Seven mixtures of the gases were used, viz. 0, 1, 2, 3, 4, and 5 per cent. oxygen, and air. Six experiments were carried out from each of which one of the mixtures 0–5 per cent. oxygen was omitted, transport in air being carried out on all occasions in order that some idea of the variability between occasions could be obtained. Eighteen shoots were used on each occasion, three being assigned at random to each of the gas mixtures; the figures in Tables III and IV for transport rate and amounts of auxin transported are the averages of the three shoots.

Results

Table III shows the rate of movement of auxin in cm./hr. as determined from the number of stem sections evacuated during the storage period, while Table IV shows the amount of auxin transported during the storage period expressed as a percentage of the average amount transported in the three shoots that were stored in air, thereby overcoming to some extent the variability between occasion of test; each figure represents the mean of three shoots.

These data show clearly that the rate of transport and amount of auxin transported are both strongly influenced by the oxygen concentration to which the stem is exposed. Only very little movement occurs under anaerobic conditions. There is a steady increase in the rate of movement, to 0·46 cm./hr. at 5 per cent. oxygen, but this is well below the figure of 0·70 cm./hr. obtained for normal aerobic conditions. This figure compares favourably with the figure obtained in the temperature experiments where a rate of 0·74 cm./hr. was obtained at 27° C.

A similar effect is shown for the amount of auxin transported, small amounts passing even under anaerobic conditions, but about four times as much is moved at 5 per cent. oxygen, and an even greater amount in air. The very

TABLE III

Effect of Oxygen Concentration on Rate of Transport of Auxin (cm./hr.) at 25° C.

Test number.	% oxygen. 0.	1.	2.	3.	4.	5.	Air.
295	0	0·05	0·05	0·22	0·22	—	0·72
298	0·11	0·05	0·28	—	0·44	0·33	0·72
301	0	—	0·17	0·28	0·28	0·39	0·67
303	0	0·17	0·28	0·39	—	0·50	0·67
304	—	0·17	0·22	0·33	0·33	0·39	0·72
306	0·11	0·17	—	0·33	0·39	0·67	0·67
Mean	0·04	0·12	0·20	0·31	0·33	0·46	0·70

close relation holding between the amount of auxin transported and the rate of transport can be seen from Fig. 4, in which the average figures for rate and amount transported are plotted against oxygen concentration. In both cases the relation with oxygen concentration is approximately linear.

TABLE IV

Effect of Oxygen Concentration on the Amount of Auxin transported at 25° C. expressed as a Percentage of that transported in Air

Test number.	% oxygen. 0.	1.	2.	3.	4.	5.
295	29	18	48	65	85	—
298	27	27	44	—	59	63
301	21	—	93	58	101	110
303	18	12	27	47	—	125
304	—	21	6	13	26	31
306	10	31	—	69	98	99
Mean	21	22	44	50	74	86

The total amounts of auxin obtained from the stem segments were very variable and made comparisons between treatments difficult. To overcome some of the variability between tests the auxin obtained from each shoot has been expressed as a percentage of the amount obtained from the three air-stored shoots on each occasion, and average values for each treatment are presented in Table V.

There would appear to be no effect of oxygen concentration down to 2 per cent. oxygen upon the amount of auxin which is obtained from a given length of tissue, but below 2 per cent. there may possibly be a very small reduction. Auxin is thus not readily destroyed under anaerobic conditions.

TABLE V

Total Auxin as Percentage of Average Amount obtained from Air-stored Segments

Test number.	% oxygen. 0.	1.	2.	3.	4.	5.
295 . .	95	78	107	107	114	—
298 . .	70	76	99	—	83	90
301 . .	93	—	125	99	138	120
303 . .	94	82	85	82	—	161
304 . .	—	96	74	66	81*	77
306 . .	62	82	—	95	120	84
Mean .	83	83	98	90	107	106

* Only one shoot was used to give this figure; all other values are means of three shoots.

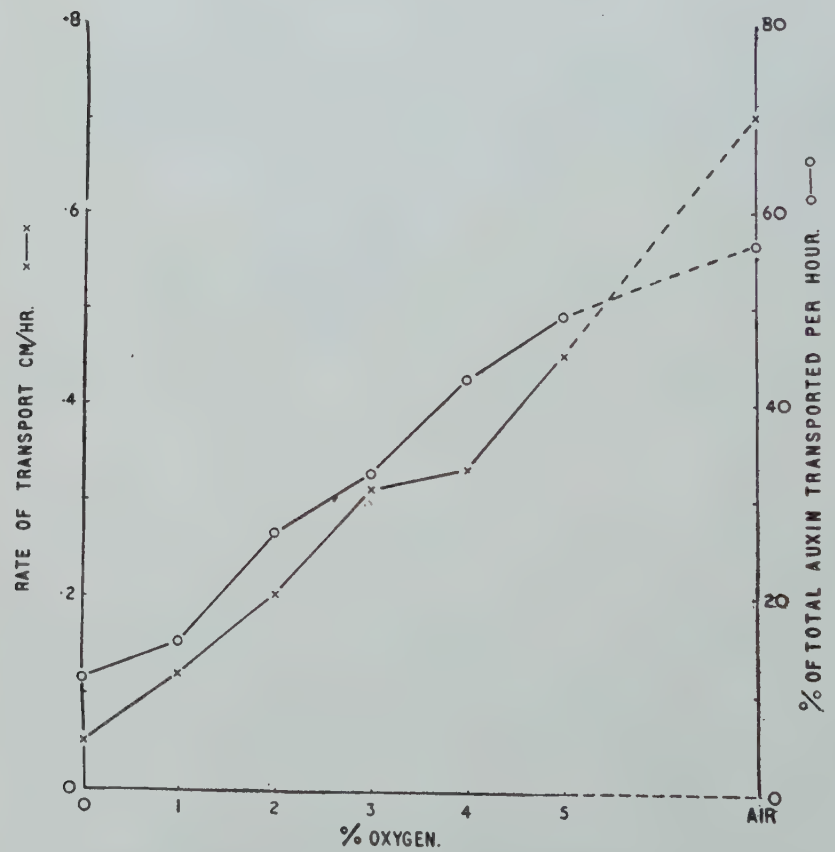

FIG. 4. The effect of oxygen concentration upon the rate of transport of auxin and the total auxin transported.

Discussion

The preliminary experiments carried out by Hatcher (unpublished) were not conducted under controlled conditions, but the values obtained, namely 0·5–0·75 cm./hr., agree tolerably well with the work here reported, assuming a room temperature of 17°–22° C. The present work has extended the investigation using a wide range of controlled temperatures. Two important effects of temperature are established: (1) an increase in transport rate from 0° C. to 27° C. with a Q_{10} of approximately 2; (2) an equally marked increase in amount of auxin transported over the range 0°–32° C. In both these cases a marked optimum appears, and above 32° C. transport rate and amount transported decline, reaching zero at 42° C. This decline at supra-optimal temperatures is, at least in part, due to disappearance of auxin in the stem tissues either by destruction, inactivation, or inhibitor production. These findings conflict with the conclusion reached by van der Weij (1932) in the study of auxin transport in the coleoptile. He stated: 'the rate of growth substance transport in the coleoptile is, in all probability, quite independent of the temperature' (p. 480, § 4). So far as 'intensity' of transport is concerned, meaning by this the quantity of auxin transported, an optimum curve was obtained very similar to that shown in Fig. 3 in this paper with a pronounced maximum at 30° C. followed by a rapid decline. To account for this apparent paradox van der Weij suggested a model based upon transport on a moving band. The rate of movement of the band is independent of temperature but the 'width or capacity' of the band is temperature controlled (p. 482). The conclusion that temperature is without effect was based upon the interpretation of curves relating quantity of auxin transported at different temperatures and the time over which the auxin was collected as it emerged from the base of the coleoptile segment. The crucial point in the argument is that curves for various temperatures, all straight lines, when extrapolated meet at a single point on the time axis. The time interval of this moment from zero time was regarded as the time taken for auxin to appear at the lower surface; and this time was independent of the prevailing temperature. Instead of fitting straight lines to the experimental points by eye as was done by van der Weij, lines of closest fit were calculated from van der Weij's data by Dr. C. L. Mer, and the results obtained are shown in Fig. 5 below. The point *X* on the abscissa is the point to which van der Weij made all the lines which he drew through the points converge. It is clear that an objective representation of the experimental results does not support the contention that all the lines regress to a single point, and indeed the indication is that auxin arrived at the base of the section sooner at higher than at lower temperatures. The conclusions of van der Weij that temperature is without effect on rate of auxin transport is thus not well founded.

In the work presented here the contrary conclusion has been established. Both the rate of transport and the 'intensity' in van der Weij's terminology

are equally dependent upon temperature and show very similar temperature relations.

The investigation of the influence of oxygen tension upon auxin transport has established two similar effects, namely (1) a linear relationship between oxygen tension and transport rate, and (2) a similar relationship with the

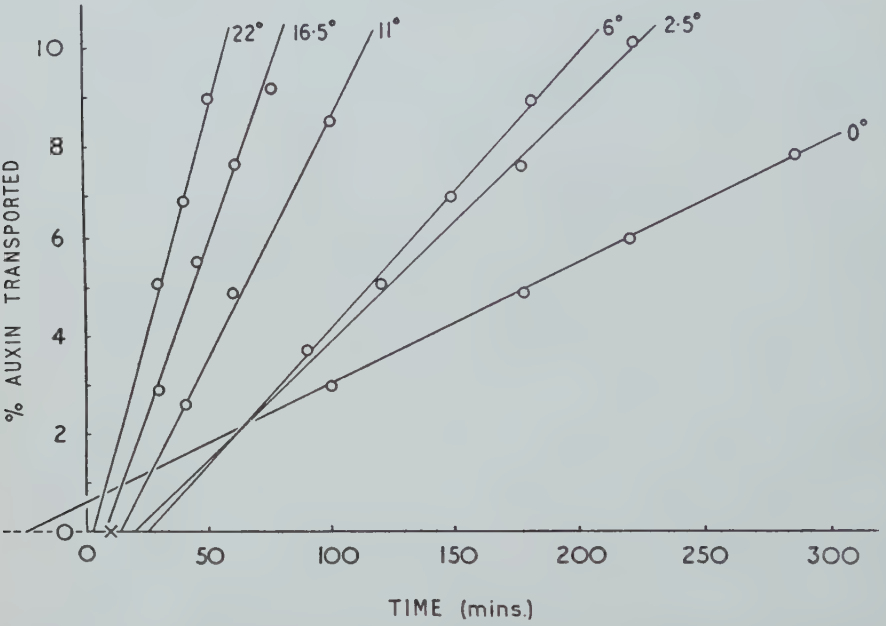

FIG. 5. Lines of closest fit to the original points obtained by van der Weij for the amounts of auxin transported after various time intervals. The lines drawn by van der Weij through the points converged at *X*, van der Weij 1932, Fig. 22. Exp. 124, p. 468.

amount of auxin transported. Du Buy and Olson (1940) observed that the amount of auxin transported through a 5-mm. section of *Avena* coleoptile from an auxin source at its upper end to a plain agar block at the base was greatly reduced if the sections were first treated either with N/3000 KCN or with dinitrophenol at 10 and 100 p.p.m. but not with 1 p.p.m. DNP. These effects are similar to those exerted by these substances upon respiration. The results obtained in the work described in this paper would support the theory of a relationship between respiration and auxin transport.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. E. S. J. Hatcher for making available unpublished data, and to Dr. C. L. Mer for the data used in preparing Fig. 5; also to Dr. F. R. Tubbs, Director of East Malling Research Station where the work was carried out, and to other members of the staff for assistance in various ways.

Literature Cited

Avery, G. S., Birkholder, P. R., and Creighton, H. B., 1937: Production and Distribution of Growth Hormone in Shoots of *Aesculus* and *Malus*, and its Probable Role in stimulating Cambial Activity. Amer. J. Bot., xxiv. 51–58.

Boysen Jensen, P., 1936: Growth Hormones in Plants. McGraw-Hill Book Co. New York.

Buy, H. G. Du., and Nuernbergk, E., 1932: Phototropismus und Wachstum der Pflanzen. Ergeb. der. Biol., ix. 358–544.

—— and Olson, R. A., 1940: The Relation between Respiration, Protoplasmic Streaming and Auxin Transport in the *Avena* Coleoptile, using a Polarographic Microrespirometer. Amer. J. Bot., xxvii. 401–13.

Clark, W. G., 1937: Electrical Polarity and Auxin Transport. Plant Physiol., xii. 409–40.

Dolk, H. E., 1929: Über die Wirkung der Schwerkraft auf Koleoptilen von Avena sativa. Proc. kon. Acad. Wetensch. Amst., xxxii. 40–47, 1127–40.

—— 1936: Geotropism and the Growth Substance. Rec. Trav. Bot. Neerl., xxxiii. 509–85.

Hatcher, E. S. J., 1948: The Study of Auxin in Shoots of Apple and Plum. Ann. Rep. East Malling Res. Sta. for 1947, 113–16.

Jacobs, Wm. P., 1950: Auxin-transport in the Hypocotyl of *Phaseolus vulgaris* L. Amer. J. Bot., xxxvii. 248–54.

—— 1951: Auxin Relationships in an Intercalary Meristem: Further Studies on the Gynophore of *Arachis hypogaea* L. Ibid., xxxviii. 307–10.

—— 1952: The Role of Auxin in Differentiation of Xylem around a Wound. Ibid., xxxix. 301–9.

Keyworth, W. G., 1951: A Petri-dish Moist Chamber. Trans. Brit. mycol. Soc., xxxiv. 291–2.

Oserkowsky, J., 1942: Polar and Apolar Transport of Auxin in Woody Stems. Amer. J. Bot., xxix. 858–66.

Rawes, R., and Hatcher, E. S. J., 1949: A Method for estimating Hormone Activity in the Plant. Ann. Rep. East Malling Res. Sta. for 1948, 157–9.

Skoog, F., 1938: Absorption and Translocation of Auxin. Amer. J. Bot., xxv. 361–72.

Thimann, K. V., and Skoog, F., 1933: Studies on the Growth Hormone of Plants. III. The Inhibiting Action of the Growth Substance on Bud Development. Proc. Nat. Akad. Sc., xix. 714–16.

—— 1934: Studies on the Growth Hormone of Plants. VI. The Distribution of the Growth Substance in Plant Tissue. J. Gen. Physiol., xviii. 23–34.

Weij, H. G. van der, 1932: Der Mechanismus des Wuchsstoff-transportes. Rec. Trav. Bot. Neerl., xxix. 379–496.

Went, F. W., 1926: On Growth accelerating Substances in the Coleoptile of *Avena sativa*. Proc. Kon. Akad. Wetensch. Amst., xxx. 10–19.

—— 1928: Wuchsstoff und Wachstum. Rec. Trav. Bot. Neerl., xxv. 1–116.

—— and Thimann, K. V., 1937: Phytohormones. Macmillan, New York.

—— 1939: Transport of Inorganic Ions in Polar Plant Tissues. Plant Physiol., xiv. 365–9.

—— and White, R., 1939: Experiments on the Transport of Auxin. Bot. Gaz., c. 465–84.

The Stamen Patterns of Cultivated Plums

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With eight Figures in the Text

Abstract

Stamen numbers were counted from flowers of cultivated plum varieties (*P. domestica*), seedling clones from crosses between varieties, and six other *Prunus* species. Varieties have a wider range of stamen number than the presumed parental species, *P. spinosa* and *P. cerasifera*, and extend from 15·3 to 33·0 stamens.

Counts on varietal and seedling clones indicate genetical control of stamen pattern; clones have uniform patterns, but differ from one another. Annual climatic variations affect stamen number, with varieties reacting differently. There are relationships between stamen numbers and some economic characters. Stamen patterns may be helpful as an additional diagnostic character and for the identification of bud-sports.

Introduction

THE androecium is two, three, or four times as many as the petals, or infinite, in Rosaceae generally, according to Willis (1919). Stamen number is a character not previously used by taxonomists in classifying the major groups of Rosaceae, yet it is now known that Prunoideae and Pomoideae differ widely in this property. Previous studies of stamen numbers and variation in cultivated cherries and pears by Haskell (1954 *a* and *b*) have demonstrated a wide range of variation in the former and comparative stability in the latter; these studies have proved useful not only for revealing such differences in stamen numbers but also as a help for diagnostic purposes. Furthermore, the results showed that in the Rosaceae there are even more differences between the primary chromosome group Prunoideae ($x = 8$) and the secondary group Pomoideae ($x = 17$) than the characteristics of their fruits and other morphological differences already reported by Moffett (1931).

Cultivated European plums (*Prunus domestica*) are hexaploids ($2n = 48$), and $x = 8$ is the basic number for the genus *Prunus* (Mather, 1937). According to Crane (1950) the European plum has probably arisen from natural hybridization between diploid cherry plums (*P. cerasifera* syn. *P. divaricata*, $2n = 16$) and tetraploid sloes (*P. spinosa*, $2n = 32$). Such hybrids are triploids with $2n = 24$; they are sterile and have been found growing naturally in the North Caucasus by Rybin (in Crane and Lawrence, 1952). The hexaploid cultivated plums could have arisen by sudden doubling of chromosomes

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]

in the triploid hybrid vegetative shoots. More likely they resulted from fusion of unreduced gametes from both the male and female parental species, for unreduced gametes are widely known in various genera of the Rosaceae. The gage plums differ somewhat in growth, fruit characters, and flavour from garden plums, and may possibly have come from crossing between *P. domestica* and *P. insititia* (Taylor, 1949). They are here included with the *domestica* group, with which they are compatible.

It was considered, therefore, worth extending the stamen survey to plums to see whether they show the wide range of variation in stamen number found in cherries (*Prunus avium*) or the constancy of pears (*Pyrus communis*). In addition, it was hoped that these studies would shed further light on the origins of plums, besides having some possible diagnostic value.

Historical Survey

Cultivated plums normally have five petals per flower, although there are a few varieties with extra petals which really are petalloid stamens. In his study on the morphology of plum flowers Dahl (1935) has stated that there are 20 to 30 stamens per flower in three whorls; these consist of 10 in the outer whorl and 5–10 stamens in each of the two inner whorls. It was not stated, however, whether there was variation between named varieties. Furthermore, Dermine and Liard (1953) when identifying and describing modern varieties considered stamen number to have no importance as a diagnostic feature: hence they failed to give any counts of stamen number. Cullinan (1937), in his detailed account of the improvement of the stone fruits generally, omits specific mention of stamen number, although Hatton (1920) had included it as a character for identifying plum rootstocks.

As Table I shows, however, numerous authors have recorded what they considered the stamen numbers to be in various *Prunus* species. This table reveals that there is not only considerable variation between species but also between observations of different authors on the same species. Indeed, the same authors vary in their own observations; for example, Hedrick (1925) stated that the stamen range for the genus *Prunus* is only 15–20, arranged in three whorls, whereas earlier in 1911 when describing *P. domestica* (and two other species) he gave the stamen number as about 30. Waugh (1901) has given 15–30 as the range in the genus *Prunus*, and Rendle (1925) gives 10, 20, or more stamens for the sub-family Prunoideae, but does not make further sub-division. In the British flora Bentham and Hooker (1908) have described the number for the genus *Prunus* as numerous, while Clapham, Tutin, and Warburg (1952) more recently have said that the number is about 20. It is clear, therefore, that a detailed survey of stamen number in the cultivated plum and related species would help clarify these conflicting reports.

Materials

The Institution's collection of plums at Bayfordbury was used. A large number of named varieties in the *domestica* group was included for study and

seven families of unnamed seedlings from several crosses between them. Four other *Prunus* species were examined, including the related *P. insititia*, *P. cerasifera*, and *P. spinosa*. Most counts were made on pot trees in 1952, 1953, and 1954; a few counts were taken in 1952 from pot trees in the glasshouse, which had been transferred from the frameyard in January or February. All the seedlings were counted in the field and also some varieties. Ten flowers were picked at random from each tree, as this had previously been found

TABLE I

Literature on Stamen Numbers in Plums and Peaches

Prunus species.	Chromosome number.	Stamen number.	Number of whorls.	Author.
P. spinosa	32	*c.* 12–15	—	Hutchinson, 1945
P. insititia	48	*c.* 25	—	Hedrick, 1911
P. domestica	48	*c.* 30	—	,, 1911
,,		20–30	1 with 10 st., 2 with 5–10 st.	Dahl, 1935
,,		15–20	—	Mace, 1949
,,		30	3	Taylor, 1949
,,		not stated	—	Dermine and Liard, 1953
P. americana	16	*c.* 30	—	Hedrick, 1911
P. hortulana	16	*c.* 20	—	,, 1911
P. persica	16	20–30	—	,, 1917
P. triflora	16	*c.* 25	—	,, 1911
P. padus (syn. *Padus racemosa*)	32	∞	—	Hutchinson, 1950
P. laurocerasus L. (syn. *Laurocerasus officianalis*)	*c.* 176	20	2	,, 1950
P. curdica	Unknown	*c.* 20	—	Hedrick, 1911
P. monticola	,,	30+	—	,, 1911
P. munsoniana	,,	*c.* 20	—	,, 1911

satisfactory for cherries (Haskell, 1954 *a*). When possible, duplicate stamen counts were made on several trees of the same clone, and of trees in pots and in the field. Flowering time of plums in England extends from March to early May.

Mean stamen numbers and their standard deviations (S.D.) were determined and the coefficients of variation (*c*) calculated. These, together with the modal values for each variety, are given in Table II; classification is by years and by growing conditions. Counts of stamen numbers in some related and other species of *Prunus*, including a few peaches (*P. persica*), are given in Table III.

HEREDITARY INFLUENCES

Several analyses were made to see whether stamen numbers and their variation within a variety are influenced by heredity.

Clonal variation. Table IV demonstrates how trees vary within clones

TABLE II

Stamen Numbers and Variations in Cultivated Hexaploid European Plums (P. domestica 2 n = 48)

Variety.	Mode.	Mean.	S.D.	C.
1. *Pots in glasshouse* 1952.				
Victoria	—	23·5	±1·84	7·83
Oullin's Golden Gage	26	25·9	±1·20	4·63
Denniston's Superb	26, 27	26·6	±1·95	7·33
Marjorie's Seedling	—	26·9	±1·91	7·10
Coe's Golden Drop	30	28·2	±2·30	8·16
Golden Transparent	30	29·2	±1·39	4·76
Allgrove's Superb	30	30·2	±0·92	3·05
Jefferson	—	31·0	±1·33	4·29
Mean		27·7		5·89
2. *Pots in frameyard* 1952.				
Allgrove's Early Greengage	18, 25	20·7	±3·25	15·70
Pershore	23	23·2	±1·62	6·98
Pojegatche	22	23·2	±1·81	7·80
Early Rivers	—	23·5	±2·01	8·55
Old Greengage	—	24·9	±3·03	12·17
President	26	25·3	±1·77	7·00
Oullin's Golden Gage	28	26·2	±1·75	6·68
Brandy Gage	28	26·8	±2·25	8·39
Red Winter	—	27·1	±2·18	8·04
Olympia	—	28·2	±2·35	8·33
Golden Transparent	—	29·8	±1·40	4·70
Mean		25·4		8·58
3. *Pots in frameyard* 1953.				
Keen of Bosnia	16	15·3	±2·06	13·46
Late Orange	—	22·3	±1·70	7·62
Warwickshire Drooper	21	22·3	±1·49	6·68
Purple Pershore	—	22·7	±2·16	9·51
Purple Pershore	22, 23	22·8	±0·78	3·42
Black Prince	—	23·0	±2·00	8·69
Belle de Septembre	23, 24	23·8	±0·79	3·32
Cropper	—	23·9	±2·47	10·33
Delicious	—	24·5	±1·58	6·45
Paulkowo	—	24·5	±1·96	8·00
Victoria	23	24·5	±1·90	7·75
Marjorie's Seedling	24	24·7	±1·42	5·75
Blue Tit	25, 26	24·8	±1·75	7·06
Bountiful	—	25·3	±2·40	9·49
Early Rivers	25	25·4	±1·17	4·61
Comte D'Althan	—	25·5	±2·41	9·45
Supreme	25	25·6	±1·58	6·17
Bryanston Gage	—	25·7	±1·94	7·55
Pojegatche	26	25·7	±1·63	6·34
Blackbird	—	26·0	±1·70	6·54
Pershore	—	26·1	±1·52	5·82
Early Rivers	—	26·3	±2·31	8·78
Czar	27	26·6	±1·07	4·02
Brahy's Greengage	26, 27	26·7	±1·42	5·31
Brandy Gage	—	26·7	±1·92	7·19

TABLE II (*continued*)

Variety.	Mode.	Mean.	S.D.	*C.*
Jefferson	—	26·7	±3·06	11·46
Belgian Purple	27, 28	26·8	±1·75	6·53
Cambridge Gage	26	26·9	±2·08	7·73
Denniston's Superb	—	27·0	±2·54	9·41
Jubilee	27	27·0	±1·49	5·52
President	—	27·0	±2·45	9·07
Reine Claude de Bavay	26	27·0	±1·49	5·51
Belle de Louvain	—	27·1	±2·38	8·78
Old Greengage B	28	27·3	±2·16	7·91
Golden Transparent	—	27·4	±2·59	9·45
Blue Rock α	28, 29	27·6	±1·35	4·89
Old Greengage C	28	28·1	±1·28	4·55
Greengage (own roots)	27, 29	28·4	±1·17	4·12
Lawson's Golden Gage	29, 30	28·5	±1·58	5·54
Pond's Seedling	—	28·6	±2·46	8·60
Allgrove's Old Greengage	28, 30	28·8	±1·13	3·92
Kirke's Blue	29	28·8	±1·62	5·62
Old Greengage A	28	28·8	±1·23	4·27
McLaughlin's Gage	30	28·9	±1·91	6·61
Thorn Sweet	28, 29	28·9	±2·85	9·86
Early Transparent	30	29·2	±1·23	4·21
Old Greengage D	28	29·3	±1·25	4·27
Blue Rock β	30	29·5	±0·97	3·29
Transparent Gage	30	30·2	±1·48	4·90
Utility	30	30·6	±0·84	2·74
Allgrove's Superb	30, 31	30·8	±0·79	2·56
Primate	33	33·0	±1·49	4·51
Mean		26·5		6·64
4. *Pots in frameyard* 1954.				
Early Rivers	20	20·1	±1·28	6·37
Early Rivers	—	23·3	±1·70	7·30
Victoria	22	23·3	±1·56	6·69
Blue Rock β	—	25·1	±2·21	8·80
Denniston's Superb	—	25·8	±2·46	9·53
Early Laxton	25, 26	26·0	±1·33	5·11
Blue Rock α	—	26·6	±2·04	7·67
Allgrove's Superb	31	30·1	±1·91	6·34
Mean		25·0		7·23
5. *Trees in field* 1954.				
Victoria	23	23·2	±1·14	4·91
Blue Rock	26, 27	25·0	±3·20	12·80
President	—	25·0	±1·49	5·96
Maynard	24, 28	25·6	±2·12	8·28
Denniston's Superb	27	26·0	±1·63	6·27
Prince of Wales	—	27·1	±1·37	5·05
Blue Rock	27	27·8	±1·93	6·94
Allgrove's Superb	30	29·5	±1·18	4·00
Early Transparent	—	29·6	±1·50	5·07
Allgrove's Superb	30	30·0	±0·46	1·53
Utility	—	30·4	±1·58	5·20
Golden Transparent	31	31·5	±1·84	5·84
Mean		27·6		5·99

which are genetically identical and between clones which are genetically different. It is clear that specific clones have their own patterns of variation, which includes a specific mean value and typical range of internal variation; hence these must be a genetic property. The two values could be useful additional diagnostic features in plums, if little variation in mean number is

TABLE III

Mean Stamen Numbers within Prunus Species other than P. domestica

Species.	Name.	2*n*.	Year.	Mean stamen number.	S.D.	*C*.	$\bar{C}$.
P. cerasifera (syn. *P. divaricata*)	Cherry plum	16					
Myrobalan B			1952	26·0	±2·2	8·5	
			,,	21·0	±3·0	14·4	
			1954	25·3	±1·7	6·7	
Myrobalan			,,	28·1†	±1·6	5·7	
Red Myrobalan			,,	23·6	±1·8	7·5	
							8·6
P. persica	Peach	16	1953	29·2	±2·3	7·7	
			,,	33·9	±1·0	2·8	
P. persica var. *alba plena*			,,	40·2	±2·5	6·3	5·6
P. spinosa	Sloe	32	1953	14·8	±1·6	10·5	
			1954	16·2†	±1·8	11·2	
			,,	19·5F	±2·1	10·9	
			,,	19·4F	±1·4	7·4	
			,,	19·9F	±2·3	11·7	
							10·3
P. insititia	Damson	48					
Julien B			1953	19·9	±1·5	7·3	
Merryweather			,,	25·6†	±2·1	8·3	
			1954	28·0	±1·3	4·8	
Langley Bullace			1953	25·9	±2·4	9·3	
							7·4
P. laurocerasus		*c.* 176	1952	19·5	±1·4	7·3	7·3
P. cocomilia		?	1953	22·1	±1·5	6·9	6·9

† = Trees in pots in glasshouse.
F = Trees in the field.
All other counts from pot trees in the frameyard.

to be expected between trees of the same clone which have the same genetic constitution. However, counts from two trees of Myrobalan B,[1] widely used as a rootstock, are included in Table IV, and these appear somewhat variable. This may be because Myrobalans often are raised from seeds, so that seedlings would be morphologically similar, yet could slightly vary in their quantitative characters. Hence some variation in genetically variable Myrobalan is not altogether unexpected, although Myrobalan B itself is said to be raised from hardwood cuttings or layered shoots, which should not affect stamen number.

[1] Sometimes spelled as 'myrobolan'. We are here following the spelling in Index Londinensis and Index Kewensis.

Seedling segregations. The seed and pollen parents of the seven seedling clones were counted in the same year as the seedlings, so that comparison of parental means and means of seedlings is possible, as in Table V. The means of three seedlings from a cross of 'Early Laxton' by 'Early Transparent' are

TABLE IV

Stamen Variation within Clones of Cultivated Plums

Variety.	Year.	Number of flowers with stamens. 17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	Mean.	*C.*
Pot trees																		
Purple Pershore	1953α	—	—	—	—	—	4	4	2	—	—	—	—	—	—	—	22·8±0·8	3·4
	β	—	—	—	2	1	3	0	1	2	1	—	—	—	—	—	22·7±2·2	9·5
Early Rivers	1953α	—	—	—	—	—	—	—	2	4	3	0	1	—	—	—	25·4±1·2	4·6
(syn. Rivers Early Prolific)	β	—	—	—	—	—	—	1	2	2	0	0	3	2	—	—	26·3±2·3	8·8
Blue Rock	1953α	—	—	—	—	—	—	—	—	1	1	2	3	3	—	—	27·6±1·4	4·9
	β	—	—	—	—	—	—	—	—	—	—	—	2	2	5	1	29·5±1·0	3·3
Field trees																		
Allgrove's	1954α	—	—	—	—	—	—	—	—	—	—	—	3	1	4	2	29·5±1·2	4·0
Superb	β	—	—	—	—	—	—	—	—	—	—	—	—	1	8	1	30·0±0·5	1·5
Pot trees																		
Myrobalan B	1952α	—	—	—	—	—	1	0	2	1	1	2	2	1	—	—	26·0±2·2	8·5
	β	2	1	1	2	1	2	0	0	0	1	—	—	—	—	—	21·0±3·0	14·4

α and β refer to two trees in a clone.

lower than both parents, and the mean for a seedling of 'Early Laxton' by 'President' is also lower than the parental mean. In the cross of 'Victoria' by 'Denniston's Superb' one seedling is lower and two are higher than both the

TABLE V

Mean Stamen Numbers in Seven Selected Seedlings and Their Parents

Cross.	Parents. Male.	Parents. Female.	Parental Mean.	Hybrid selected seedlings.
Early Laxton × Early Transparent .	29·6	26·0	27·8	21·1
				21·7
				23·5
Early Laxton × President . .	25·0	26·0	25·5	23·9
Victoria × Denniston's Superb . .	26·0	23·2	24·6	23·5
				26·6
				27·1

parental mean and the value for the higher parent. This trend indicates that these genetically diverse seedlings, which had been carefully selected for their vigour and crop quality, generally show no heterosis, or very little, for stamen number.

Class of breeding system. Table VI gives the mean values of stamen numbers of varieties grouped according to Crane and Brown's (1939) classification into three pollination classes, viz. fully self-sterile (class A), mainly self-sterile

(class B), and self-fertile (class C). It is seen that there is no difference between class B and C, and although varieties in class A sometimes tend to have slightly higher values, any influence shown clearly is dominated by environmental effects.

TABLE VI

Stamen Numbers and Variation in relation to Compatibility Classes

		A (self-sterile).	B (2–5%).	C (self-compatible).	Coefficient of variation. A.	B.	C.
I	1952 glasshouse . .	29·8	—	26·4	5·17	—	6·33
II	1952 frameyard . .	25·3	—	26·4	7·00	—	6·12
III	1953 „ . .	27·9	27·5	25·6	6·95	6·11	6·51
IV	1954 „ . .	30·1	24·2	24·5	6·35	10·8	7·21
V	1954 field . . .	28·2	27·7	27·5	9·76	9·74	11·68
	Mean . . .	28·3	26·5	26·1	7·0	8·6	7·6

Bud-sports. 'Jefferson' was definitely known by its raiser to have produced 'Allgrove's Superb' as a bud-sport (Taylor, 1949). They have the same stamen numbers, viz. $31{\cdot}0 \pm 1{\cdot}3$ for 'Jefferson' and $30{\cdot}2 \pm 0{\cdot}9$ for 'Allgrove's Superb' (1952 pot trees). As this constancy is expected for trees within a clone, it is probable that stamen numbers are useful for recognizing bud-sports in plums and in other species, such as peaches, where they frequently occur. On the other hand, 'Purple Pershore', which is nowadays claimed to be a bud-sport of 'Pershore' (cf. Taylor, 1949), differs significantly from the supposed original variety: they have respectively $22{\cdot}8 \pm 0{\cdot}8$ and $26{\cdot}1 \pm 1{\cdot}5$ stamens (1953 pot trees). The resemblance of a bud-sport to its parent will depend on the type of hereditary modification involved. Hence this difference may be due either to a different type of origin of the bud-sport or to the varieties having different parentage.

ENVIRONMENTAL EFFECTS

Analyses were made of stamen numbers in relation to various environmental factors likely to affect their manifestation.

Climatic conditions. To determine whether climatic conditions affect stamen number of a variety, data of the means were assembled for six varieties counted in each of the 3 years (Table VII). On the average for these varieties there is very little annual variation and it is not consistently in any direction for all varieties. The analysis of variance, in Table VII, shows that although varieties differ significantly in their particular stamen number, there is no significant effect on it as a result of year-to-year climatic variation.

When individual analyses of variance were made on the original data for stamen patterns of 'Golden Transparent' and 'President' (Table VIII), it was found that over all 3 years there were significant differences in stamen number. For 1952–3 the differences for 'President' were highly significant, but 'Golden

TABLE VII

Annual Variation in Stamen Number

Variety.	1952.	1953.	1954.	Mean.
1. Allgrove's Superb .	30·2±0·9*	30·8±0·8	29·5±1·2F 30·0±0·5F 30·1±1·9	30·12
2. Golden Transport .	29·8±1·4	27·4±2·6	31·5±1·8F	29·57
3. Denniston's Superb .	26·6±2·0	27·0±2·5	25·8±2·5 26·0±1·6F	26·35
4. President . . .	25·3±1·8	27·0±2·5	25·0±1·5F	25·77
5. Early Rivers . .	23·5±2·0	25·4±1·2 26·3±2·3	20·1±1·3 23·3±1·7	23·72
6. Victoria . . .	23·5±1·8*	24·5±1·9	23·2±1·1F 23·3±1·6	23·63
Mean . . .	26·48	26·91	26·16	

* = Pots in glasshouse. F = Field trees. All others pot trees in frameyard.
Bracketed figures are those for trees in a clone.

Analysis of Variance

	S.S.	N.	M.S.	V.R.	*P*
Years	2·46	2	1·23	1·55	>0·20
Varieties	118·90	5	23·78	12·45	>0·001
Years—Varieties (error) . .	19·10	10	1·91		
Total	140·46	17			

TABLE VIII

Tests of Significance for Annual Variations in Stamen Patterns of two Plum Varieties

Comparison.	*P* values. Golden Transparent.	President.
Between 3 years .	<0·001	0·01
,, 1952–3 .	0·1–0·05	<0·01
,, 1952–4 .	<0·01	0·3
,, 1953–4 .	<0·001	<0·05

Transparent' showed only borderline significance; for 1952–4 'Golden Transparent' was highly significant but 'President' was not significant; and in 1953–4 'Golden Transparent' was highly significant, while 'President' was significant at the 5 per cent. level. Thus these two varieties were responding differently to environmental effects on their stamen number and variation.

Differences of rootstocks. Seven unnamed seedlings of various parentage were available for study. Each comprised a clone of 6–8 trees in the field, grafted alternately in the row on two types of rootstock, viz. 'Myrobalan' and 'Mussel'. An analysis of variance was made on data from trees of each clone.

One example is given in Table IX. No significant differences were obtained in these seedlings for the effects of rootstocks on stamen pattern within clones. It was noticed, however, that the 'remainder' items were sometimes significant, but what caused this is unknown. Thus stamen counts can be made on plum varieties regardless of which of these two stocks they are on, even though these influence the vigour and other properties of the scions.

TABLE IX

Variation in Stamen Number in Eight Trees of a Seedling Clone on two Rootstocks

Trees in clone.	Rootstock.	Number of flowers with stamens. 19.	20.	21.	22.	23.	24.	25.	26.	27.	Mean stamen number.
α	Myrobalan	—	—	2	0	2	4	1	1	—	23·5
β	Mussel	—	1	1	1	3	4	—	—	—	22·8
γ	Myrobalan	—	—	1	4	2	1	2	—	—	22·9
δ	Mussel	—	—	—	2	5	2	0	1	—	23·3
ϵ	Myrobalan	—	—	1	1	3	2	0	2	1	23·9
ξ	Mussel	—	—	3	0	3	3	1	—	—	22·9
η	Mussel	—	—	—	4	2	1	2	1	—	23·4
θ	Myrobalan	1	1	1	4	1	1	1	—	—	22·0

Analysis of Variance

	S.S.	N.	M.S.	*t* or V.R.	*P*
Between trees in clone	23·10	7	3·30	—	—
Between rootstocks	0·01	1	0·01	0·65	0·95
Remainder	23·09	6	3·84	1·63	0·20
Within trees in clone	169·29	72	2·35	—	—
Total	192·39	79	—	—	—

Pot trees and field trees. Comparisons were made in 1954 to see if stamen patterns were influenced according to whether trees grew in pots or in the field. It was found that 'Victoria' had $23{\cdot}3\pm1{\cdot}6$ and $23{\cdot}2\pm1{\cdot}1$ for a pot tree and a field tree respectively; 'Allgrove's Superb' similarly gave $30{\cdot}1\pm1{\cdot}9$ and $30{\cdot}0\pm0{\cdot}5$. Two pot trees of 'Blue Rock' gave $26{\cdot}6\pm2{\cdot}0$ and $25{\cdot}1\pm2{\cdot}2$, while two field trees of the same variety gave $27{\cdot}8\pm1{\cdot}9$ and $25{\cdot}0\pm3{\cdot}2$. Hence growing conditions which specifically affect growth of the root system do not necessarily affect stamen pattern. This agrees with the findings of Haskell (1954 *a*) that rootstocks in cherries are unimportant in this connexion.

Trees indoors and outdoors. Pot trees of two varieties were scored when grown in a glasshouse and duplicated outdoors. It was found, for example, that 'Oullin's Golden Gage' in 1952 had $25{\cdot}9\pm1{\cdot}2$ and $26{\cdot}2\pm1{\cdot}8$ stamens for a tree in the glasshouse and outside respectively. Likewise, 'Golden Transparent' in 1952 had stamen numbers of $29{\cdot}2\pm1{\cdot}4$ and $29{\cdot}8\pm1{\cdot}4$ for glasshouse and outside trees respectively. These two varieties thus strongly indicate that stamen number is not affected if trees are taken indoors in the spring; probably the stamens already have been laid down in the previous autumn, as with cherries. It is unlikely that the trees used here were in a glasshouse for two successive seasons.

Stamen Distribution

The actual frequencies of stamen number for different samples of cultivated plums grown in different years are given in Fig. 1: they may sometimes include the same varieties. From the figure it is seen that there is a wide range of stamen number per flower, i.e. from 11 to 36. Furthermore, in the three samples illustrated there is no sharp tendency to a modal value as in cherries (Haskell, 1954 *a*), although the modes are 25, 28, and 26 for 1952, 1953, and 1954 data respectively. There are minor peaks at 23 and 28 in the 1952 data, at 25 and 30 for 1953, and 20, 23, and 28 to 29 for 1954. Highest frequencies of stamens occur between 22 and 30. The peaks in the graph illustrate,

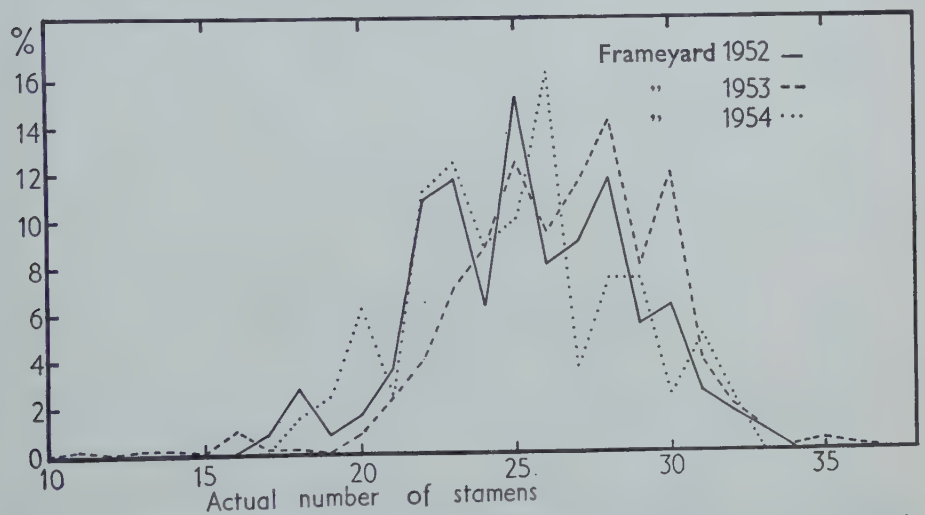


Fig. 1. Percentage frequencies of actual number of stamens per flower for various domestic plum varieties recorded in 3 years.

therefore, that stamen numbers in plums do not necessarily fall at multiples of 5, although some of the sharper peaks do. There is often a difference of 5 stamens between peaks, even when they do not fall on multiples of 5.

When histograms of frequency distributions of mean stamen number for the 1953 varieties are plotted, as in Fig. 2, it is found that most cultivated plum varieties have mean values of between 25·1 and 30·0 stamens per flower. It is also seen from Fig. 2 that if the ranges are considered for the presumed parental species of the cultivated plum, *P. spinosa* and *P. cerasifera*, there is a small group of cultivated hexaploid plums with higher stamen numbers than them. This may be due either to polyploidy, heterosis, segregation, or perhaps to a correlation with selection for larger fruit size.

Stamen Pattern in Relation to Other Characters

Several analyses were made to determine whether stamen numbers are associated with some of the more important economic characters of plums,

which may have practical importance, like flowering time. These analyses are illustrated in Figs. 3–8.

Stamen length. Mean stamen numbers of some varieties from the 1953 data were plotted in Fig. 3 against stamen lengths, as given by Dahl (1935): the mean lengths are grouped in units to the nearest millimetre. It appears that stamen numbers and stamen lengths are not necessarily correlated.

Type of fruit. Plums fall into three groups with regard to quality of their

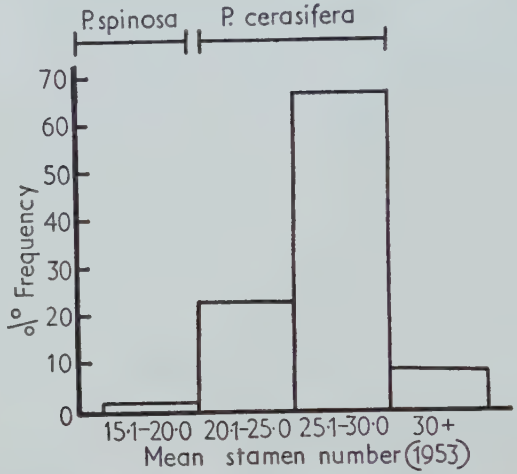


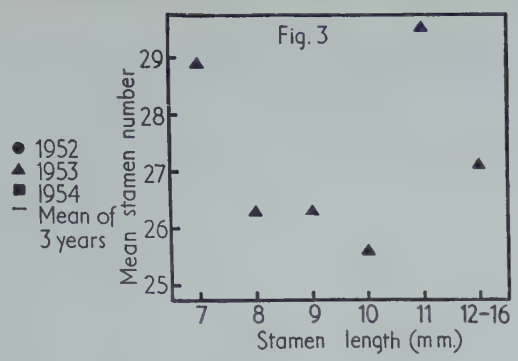
FIG. 2. Histograms of percentage frequencies for mean stamen numbers, in groups of 5 stamens, in a collection of domestic plum varieties recorded in 1953. The ranges of stamen number in the presumed parental species are also shown.

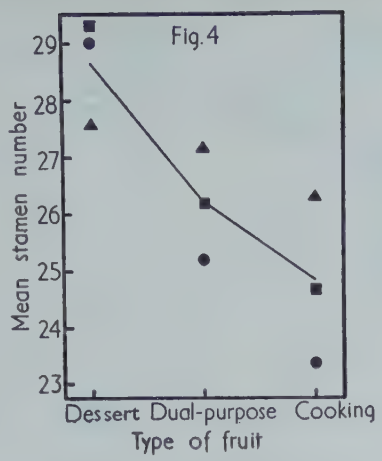
fruits. Fig. 4 shows mean stamen numbers of different varieties in 1952, 1953, and 1954 plotted against type of fruit. The best quality dessert plums are seen to have higher stamen numbers than dual-purpose and cooking plums. This is not unexpected as dessert plums often fall into class A, i.e. the self-incompatible group, which has already been shown in Table VI to manifest higher stamen numbers.

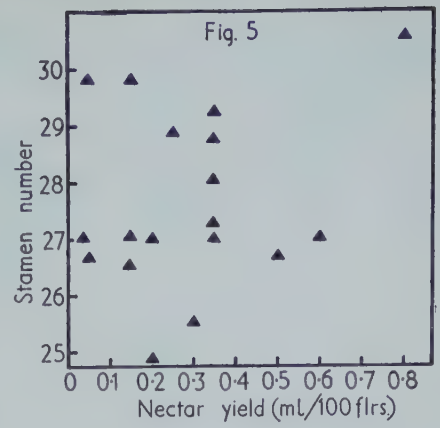
Nectar yield. Stamen numbers of individual varieties in the 1953 data are plotted in Fig. 5 against nectar secretion for those varieties as measured by Brown (1950). There is no clear-cut correlation between varieties for stamen number and nectar yield, except for 'Utility', which has both high stamen number and high nectar secretion.

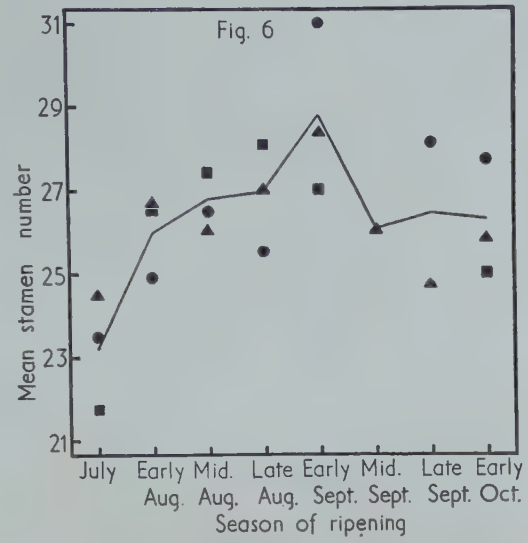
Season of ripening. Plums can be graded into eight fruit-ripening groups (Taylor, 1949). When mean stamen numbers for varieties in 1952, 1953, and 1954 are plotted against these, as in Fig. 6, it is seen that July varieties, which are the earliest to ripen, have lowest stamen numbers. The values progressively increase until early September, and then fall off slightly with latest ripening varieties.

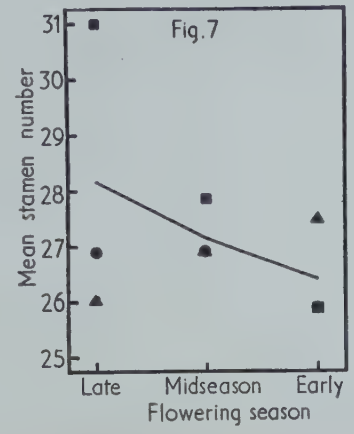
Flowering group. Plums have been grouped into three classes of flowering times to ensure successful cross-pollination in orchards. Mean stamen

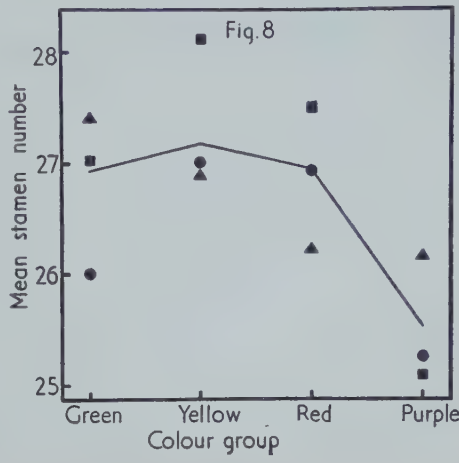












FIGS. 3–8. Mean stamen numbers plotted for six characters in the domestic plum. (Full details in the text, pages 478–80.)

numbers for varieties in 1952, 1953, and 1954 are plotted in Fig. 7 against flowering season (Crane and Brown, 1949). There is no special relation between stamen numbers and flowering time. This is not unexpected, for stamen primordia are probably laid down in the previous autumn, as in cherries.

Colour group. Colour of plums depends partly on ground or granular colour and partly on anthocyanin or sap colour, as Crane (1943) has shown. These are inherited independently to give the wide range of colours of domestic plums. Fig. 8 shows mean stamen numbers of varieties in 1952, 1953, and 1954 plotted against Taylor's (1949) colour grouping. Classification was sometimes difficult, so a variety was included in both when it overlapped two groups. It is clear from Fig. 8 that purple forms have lower stamen numbers than other colour types.

Style length. It was possible to determine mean stamen numbers of varieties according to Dahl's (1935) classification of plums by their stamen-style relationships. The three groups are: (1) those with flowers whose stamens are taller than the styles; (2) stamens and styles of equal length, and (3) stamens shorter than styles. These gave mean stamen numbers respectively of $28{\cdot}9 \pm 2{\cdot}5$, $26{\cdot}6 \pm 1{\cdot}3$, and $26{\cdot}7 \pm 1{\cdot}5$. Stamen number is not, therefore, necessarily associated with relative lengths of styles and stamens.

Internal Variation in Stamen Number

Table II records the internal variation of each variety as measured by the coefficient of variation (C). Although the varieties are classified in ascending order of stamen number, there is no corresponding scaling for C. Thus it appears that internal variation is the particular property of a variety, regardless of its actual stamen number, and depends on the genetical constitution of the clone. The range of the internal variation for 1952 pots in frameyard data is from 15·70 to 4·70, with a mean coefficient of variation of 8·58, whereas the 1952 data for cherries (pots in frameyard) ranged from 8·9 to 3·3, with a mean coefficient of variation of 6·13 (Haskell, 1954 *a*). Indeed, comparison over all cherry data with the present material shows that plums tend to have higher internal variation than cherries. This is rather unexpected as we are here dealing with hexaploid material, which might be expected to have greater internal stability than diploid cherries.

The coefficient of variation is 8·6, averaged for 3 years in the diploid *cerasifera*, whereas in the tetraploid *spinosa* it is 10·3. The mean values are a little higher than for the means of the cultivated plums, as given in Table II. Thus within plums there is a tendency to greater internal variation either with decrease in ploidy or when one approaches the wild species; this was somewhat similar in cherries. Furthermore, the internal variation in plums does not appear to be modified according to class of breeding system, as Table VI shows.

Discussion

The variation between and within plum varieties in their stamen numbers contrasts strongly with the stability of the gynoecium and other floral whorls.

The styles are constant at one per flower, although rare exceptional varieties such as 'Kirke' may have flowers with additional styles, but even then only the normal flowers set fruit. Sepals and petals are highly constant at five, although petals are variable in size and shape; in 'Grand Duke' there is a tendency regularly to produce flowers with six sepals. Very rarely the flowers on young trees are hexamerous, and tetramerous flowers are known on 'Denniston's Superb' (Dahl, 1935).

Of the environmental factors likely to produce effects, only annual climatic changes have an important influence on manifestation of stamen number, with some varieties reacting more than others. Factors such as whether trees grow in pots or in the field, and differences of rootstocks, show no effect on stamen numbers, even though they probably influence the nutriment reaching the scions.

The range of stamen numbers in the cultivated varieties of plums and the values for the presumed parental species do not detract from Rybin's hypothesis that the domestic plum has arisen from *P. spinosa* crossing with *P. divaricata*, followed by chromosome doubling. Indeed, there is general confirmation, as the range of variation in the values of the cultivated hexaploids is somewhat the same as that for the presumed parents. There is positive heterosis at one end of the scale, but the majority of the varieties lie within the range of the presumed parents. It seems unlikely that polyploidy has played an important role in the manifestation of stamen numbers in plums, although in strawberries Haskell and Williams (1954) have found an increase in numbers from diploid to octoploids, but a decrease in the decaploids.

Stamen numbers are not related to most of the economic characters they were tested against, except colour group, and partly to times of maturity. Now it seems that the purple colour was brought in from *P. spinosa*, whereas *P. divaricata* (= *P. cerasifera*) was red and yellow (Crane, 1943); these presumed parental species have produced a wide range of colours and shades in the domestic plum. As the mean stamen numbers in clones of *P. spinosa* that we have examined are from 14·8 to 19·9, whereas in *P. divaricata* the mean numbers are from 21·0 to 28·1 (cf. Fig. 2), it rather seems that considerable recombination must have occurred since the origin of cultivated plums. This has resulted even though low stamen number and purple colour might be expected to be linked in one of the original species. This wide range of combination of characters is readily shown in the wide range of colours and shapes obtained in plum segregations.

The unexpected difference in stamen pattern between 'Pershore' and 'Purple Pershore', which Taylor (1949) claims is a bud-sport of it, suggests that 'Purple Pershore' is not a bud-sport but may have arisen sexually, perhaps from 'Pershore' itself. Indeed, it is known also as 'Martin's Seedling', and previously in horticulture the term 'seedling' referred to a plant raised from seed. As 'Pershore' is often propagated from suckers, it would have been relatively easy for a germinating seedling to become incorporated with the true stock. Furthermore, on tracing back its history we have found that

Bagenal (1929) also thought it was a seedling, unconnected with 'Pershore', and Hooper (loc. cit.) stated it was a cross of 'Blue Diamond' and 'Rivers Early Prolific'. Thus this exception to the expected constancy in stamen pattern between a bud-sport and its original variety has revealed an error by Taylor (1949).

Crane and Brown (1939) have questioned whether the 'Old Greengage' group have originated sexually or as bud-sports. Table X lists the differences between 'Old Greengage' A, B, C, and D, together with their stamen patterns. It is clear that there is greater resemblance of A with B, and of C with D, yet A and D are alike in one characteristic, and A differs from B, C, and D in another. Furthermore, they do not differ significantly in their stamen pat-

TABLE X

Differences in the Old Greengage Group. (Based on Crane and Brown, 1939)

Character.	Greengage type. A.	B.	C.	D.
Colour of opening leaves	Green	Green	Brownish-red	Brownish-red
Flower opening	Before leaf-buds unfold	—	Intermediate between A and D	Leaves already advanced
Anther colour at dehiscence	Yellow	Almost orange with tinge of red	Like B	Like B
Fruit shape	Less oblate than C and D	Less oblate than C and D	More oblate than A and B	More oblate than A and B
Fertility: selfing	Sterile	Sterile	Partially self-incompatible	Partially self-incompatible
„ ×President pollen	Partially incompatible	Partially incompatible	Partially incompatible	Partially incompatible
„ ×Late Orange pollen	Incompatible	Partially incompatible	Partially incompatible	Incompatible
Stamen number	28·8±1·2	27·3±2·2	28·1±1·3	29·3±1·3

terns, although B and D are different while A and C are alike. This leads us to suspect that they have all arisen sexually rather than from bud-sports, as the difference in combinations for each particular character are so variable. Their likenesses would be attributable either to coming from the same mother parent or perhaps even to having both parents in common.

The indication that there is little or no heterosis for stamen number may mean that amongst modern plum varieties a state of maximum heterosis has already been reached, and little further will be gained by breeding. Crane (1950) has maintained that many vegetatively propagated crop plants, such as fruit-trees, are already kept at a high level of heterosis. On the other hand, as plums are hexaploid, the inheritance of a quantitative character like stamen number may be complicated and produce a wide range of variation that need not necessarily be a reflection of heterosis. This type of behaviour has already been found for fruit size in pears (Crane and Lewis, 1949). Furthermore, heterosis is not necessarily expected in some plum crosses in view of the rather close relationship of cultivated varieties. Indeed, it seems that in breeding from cultivated varieties there is a tendency amongst the offspring to regress back to the lower stamen number of the parental wild species, as shown by seedlings selected for trial.

The extreme group of plums which have higher stamen numbers than either of the presumed parents have moved away from values for the original wild species, and may well offer a pool of genes for future plum improvement. Those varieties with over 30 stamens per flower include the high quality plums 'Jefferson', 'Allgrove's Superb', 'Laxton's Utility', 'Transparent Gage', 'Primate', and 'Golden Transparent'. All of these come within class A of the plum groups, i.e. are self-incompatible, except 'Utility', which comes in class B and sets less than 5 per cent. on selfing.

The internal variation, as measured by the coefficient of variation, tends to run higher in cultivated plums than in cherries, in spite of the plums being hexaploids. This can also be compared with the values found in a polyploid series of strawberry flowers (Haskell and Williams, 1954) where the hexaploids had lowest internal variation and the tetraploids the highest; but there was in strawberries no clear trend in internal variation with increase in polyploidy. Thus this property of biometric internal variation of a clone still awaits clarification as to its genetic and biological significance; it may reflect the degree of inbreeding.

Finally, it is seen that the behaviour for stamen number in cultivated plums is similar to that for sweet cherries, but contrasts strongly with the constancy at 20 found in pears. In plums the range of stamen number for a variety lies between 15 and 33, whereas in cherries it is between 28 and 45. Their similarity in the extent of their range of about 18 stamens is remarkable, but plums have lower numbers than cherries and there is little overlapping of the ranges. There is further evidence, therefore, not only for a difference between the Prunoideae and Pomoideae, but also in the variation of a quantitative character such as stamen pattern within the Prunoideae.

Summary

1. Stamen numbers and variation were determined in cultivated varieties and seedling clones of hexaploid European plums (*P. domestica*) and in some other *Prunus* species. Stamen patterns differ between varieties, but are uniform within a clone. One genuine bud-sport had the same number as its parents, while another presumed bud-sport did not; historical evidence showed it had been sexually produced.

2. Seedlings especially selected for vigour and fruit quality show little heterosis in stamen number compared with the parents. Self-incompatible plums have higher mean stamen numbers than those partly and wholly compatible.

3. Stamen pattern is unaffected by growing conditions or by rootstocks. There is annual variation, in which varieties respond differently.

4. Most varieties have mean stamen numbers between 25·1 and 30·0. A small group have over 30, being higher than either of the presumed parents (*P. spinosa* and *P. cerasifera*). Plums (hexaploids) generally have higher internal variations than cherries (diploids).

5. There is no relation between stamen number and flowering season,

stamen length, nectar yield, or style length, but purple varieties have lower numbers than other colour groups. Dessert (mainly self-incompatible) plums have higher numbers than dual purpose and cooking varieties.

6. The range of variation in stamen patterns of plums agrees with earlier findings in cherries. The Prunoideae are variable in this character, in contrast to the relative constancy of Pomoideae. Stamen patterns may be helpful as an additional diagnostic character in plums.

We wish to thank Mr. A. G. Brown for his interest in and support of this study.

Literature Cited

Bagenal, N. B., 1929: Purple Pershore Plum. In The Fruit Grower of Jan. 24.

Bentham, G., and Hooker, Sir J. D., 1908: Handbook of the British Flora. 6th edn. London.

Brown, A. G., 1950: Factors affecting Fruit Production in Plums. R.H.S. Fruit Year Book, London, pp. 12–18.

Clapham, A. R., Tutin, J. G., and Warburg, E. F., 1952: Flora of the British Isles. Cambridge.

Crane, M. B., 1943: Cultivated Plants of the Past, Present and Future. Endeavour, ii. 111–16.

—— 1950: The Origin and Improvement of Cultivated Plants. J. Roy. Hort. Soc., lxxv. 427–35, 465–74.

—— and Brown, A. G., 1939: Incompatibility and Sterility in the Gage and Dessert Plums. J. Hort. Sci., xvii. 51–66.

—— —— 1949: The Fertility Rules in Fruit Planting. In The Fruit, the Seed and the Soil. Edinburgh.

—— and Lawrence, W. J. C., 1952: The Genetics of Garden Plants. 4th edn. London.

—— and Lewis, D., 1949: Genetical Studies in Pears. V. Vegetative and Fruit Characters. Heredity, iii. 85–97.

Cullinan, F. P., 1937: Improvement of Stone Fruits. In Yearbook of Agriculture, U.S.D.A., pp. 665–748.

Dahl, C. G., 1935: Morphological Studies of Plum Flowers. Swedish Orchard Res. Bull., xxxviii. Malmo, pp. 1–93.

Dermine, E., and Liard, O., 1953: Identification et Description des Variétés du Prunier européen. Rev. Agric., Brux., 6me Année, ix. 1–55.

Haskell, G., 1954 *a*: Stamen Number and Variation in Diploid and Tetraploid Cherries. Ann. Bot., Lond., n.s., xviii. 95–111.

—— 1954 *b*: The Stamen Constancy of Diploid and Polyploid Pears. New Phytol., liii. 349–53.

—— and Williams, H., 1954: Biometrical Variation in Flowers of a Polyploid Series of Strawberries. J. Genet., lii. 620–30.

Hatton, R. G., 1920: Stocks for the Stone Fruits. J. Pomol., ii. 209–45.

Hedrick, U. P., 1911: The Plums of New York. New York.

—— 1917: The Peaches of New York. New York.

—— 1925: Systematic Pomology. New York.

Hutchinson, J., 1945: Common Wild Flowers. Harmondsworth, Middx.

—— 1950: Uncommon Wild Flowers. Harmondsworth, Middx.

Mace, H., 1949: Bees, Flowers and Fruit. Harlow, Essex.

Mather, K., 1937: Notes on the Cytology of Some Prunus Species. Genetica, xix. 143–52.

Moffett, A. A., 1931: A Preliminary Account of Chromosome Behaviour in the Pomoideae. J. Hort. Sci., ix. 100–10.

Rendle, A. B., 1925: The Classification of Flowering Plants, vol. ii. Cambridge.

Taylor, H. V., 1949: The Plums of England. London.

Waugh, F. A., 1901: Plums and Plum Culture. New York.

Willis, J. C., 1919: Dictionary of Flowering Plants and Ferns. 4th edn. Cambridge.

Experimental and Analytical Studies of Pteridophytes

XXIX. The Effect of Progressive Starvation on the Growth and Organization of the Shoot Apex of *Dryopteris aristata* Druce

BY

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With eighteen Figures in the Text

Abstract

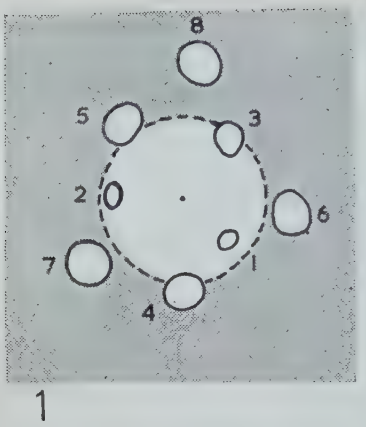
Observations on shoot apices of *Dryopteris aristata* maintained under conditions of progressive starvation for periods of up to a year are recorded. Changes in the size of the shoot apex and leaf primordia, in the rates of inception and development of leaf primordia, and in phyllotaxis, are described and discussed.

Introduction

DURING the growth of a plant to maturity the volume of its apical meristem gradually increases and finally attains a relatively constant size (e.g. Wardlaw, 1948, 1952). Conversely, starvation causes a decrease in size of the apical meristem. The cultural conditions under which apices of *Dryopteris aristata* Druce (*D. austriaca* (Jacq.) Woynar) are kept for the purposes of surgical experiments eventually bring about a progressive decrease in the size of the shoot apex; it is not known whether this is due to a lack of mineral nutrients or of substances normally supplied by the older leaves, or to a failure of the mechanism of synthesis in the meristem.

Comparatively few adequate ontogenetic studies of the shoot apex in vascular plants have been made, although the existence of interesting developmental differences between juvenile and mature plants has long been known. Steeves and Wetmore (1953) have recently discussed the different rates of development of leaf primordia in sporelings and mature plants of *Osmunda cinnamomea*, thus drawing attention to the need for a study of the ontogeny of the apical bud. The importance of nutrition in plant ontogeny has recently been emphasized by Allsopp (1953, 1953*a*, 1954), who has shown, by induced reversion and other experiments, that nutrition is one of the factors which control the heteroblastic development of leaves in *Marsilea*. He considers that the primary effect is upon the size of the shoot apex, this in turn affecting leaf development, since it may be expected that a leaf borne on a large apex could undergo a longer period of development. It is of interest, therefore, to inquire into the effect of lowering the nutritional status of the shoot apex of *Dryopteris aristata* by prolonged maintenance under unfavourable conditions, upon the growth, organization, and formative activities of the apical meristem.

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]



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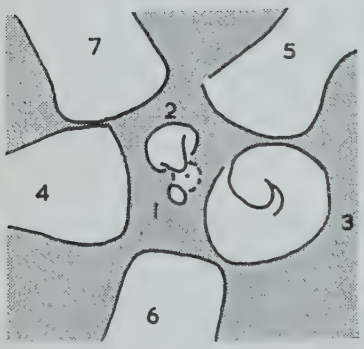

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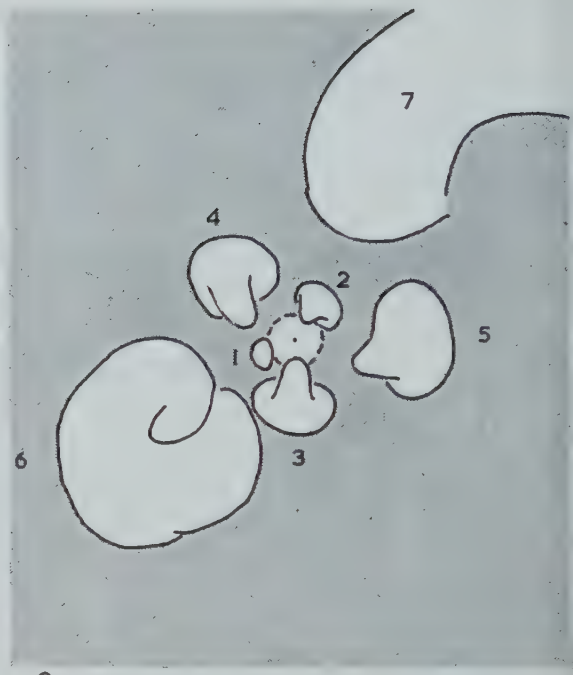

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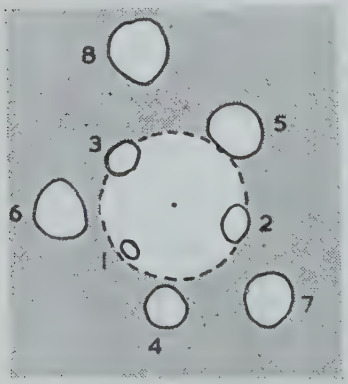
FIGS. 1–3. Expt. 4. Surface views of the shoot apex; Fig. 1, 0 days; Fig. 2, 123 days, and Fig. 3, 170 days from the beginning of the experiment. Note the decrease in size of the apex and the increase in size and morphological complexity of the leaf primordia. (Apical cone indicated by the broken line; leaf primordia numbered afresh in each drawing.) (×24.)



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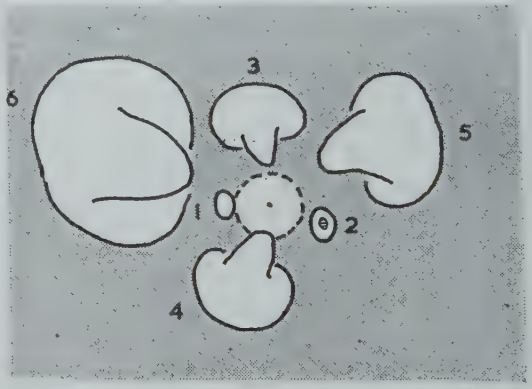

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1 mm.

FIGS. 4–5. Expt. 6. Surface views of the shoot apex; Fig. 4, 0 days and Fig. 5, 116 days after the beginning of the experiment. Note the decrease in size of the apex and the increase in size and morphological complexity of the leaf primordia (cf. Figs. 1–3). (× 24.)

Materials and Methods

Large shoot apices of *Dryopteris aristata* borne on approximately cubical blocks of rhizome, with a side of 2·5–3 cm., were laid bare as described in earlier papers in this series (e.g. Wardlaw, 1944), and maintained in the laboratory in pans of moist peat with no other source of mineral nutrients. Four specimens were kept under observation for 9 weeks, allowed to grow on without disturbance for 5 weeks more, and then kept under approximately weekly observation for further extensive periods of up to a year (expts. 1–4). Five specimens were kept under observation for 6 weeks, allowed to grow on for 6 weeks, and again kept under observation for a further lengthy period (expts. 5–9). Camera lucida drawings were made at frequent intervals at a magnification of $\times 35$.

The terminology used is that of Snow and Snow (1931): visible leaf primordia are called P_1, P_2, &c., P_1 being the youngest, and the presumptive positions of new leaf primordia are called I_1, I_2, &c., I_1 being the next to arise.

Experimental Observations

A marked progressive decrease in the size of the apical cone took place during the period of observation (Figs. 1–5), the extreme result being reached in one apex, which lost its meristematic appearance and became parenchymatous after 1 year. Similar observations have been made on apices of *Onoclea sensibilis* maintained under unfavourable conditions (Wardlaw, 1945), and attenuation of apices of *Dryopteris aristata* under the specified cultural conditions has also been previously noted (Wardlaw, 1949).

Experimental apices soon recover after the initial dissection. Thereafter the rate of formation of new leaf primordia was observed to be fairly constant. After a time, however, there was a retardation of the rate of inception of new leaf primordia concomitant with a decrease in the size of the shoot apex. From Table I and Figs. 6 and 7 it can be seen that the inception of leaf primordia proceeded more rapidly during the initial period of the experiment than during the second period, and plastochrone length increased with decrease in apical size. The second period coincided with the time of year when the rate of leaf inception was falling off, and the records are open to criticism on these grounds. Accordingly, observations relating to the remaining two specimens, expts. 2 and 4, have been incorporated in Table I and Fig. 6 c. This third period coincided in part with the time of year when the rate of leaf inception is usually high, but in these specimens the rate of inception of leaf primordia still fell off. Decrease in apical size is therefore correlated with a slower rate of leaf inception. Conversely, in an ontogenetic study of maize plants Abbe and Phinney (1951) observed a progressive increase in size of the shoot apex and a rising rate of leaf inception.

Sinnott (1921) and Whaley (1939) state that, in a particular species, the size of the lateral organs is correlated with the size of the subtending apex. In the

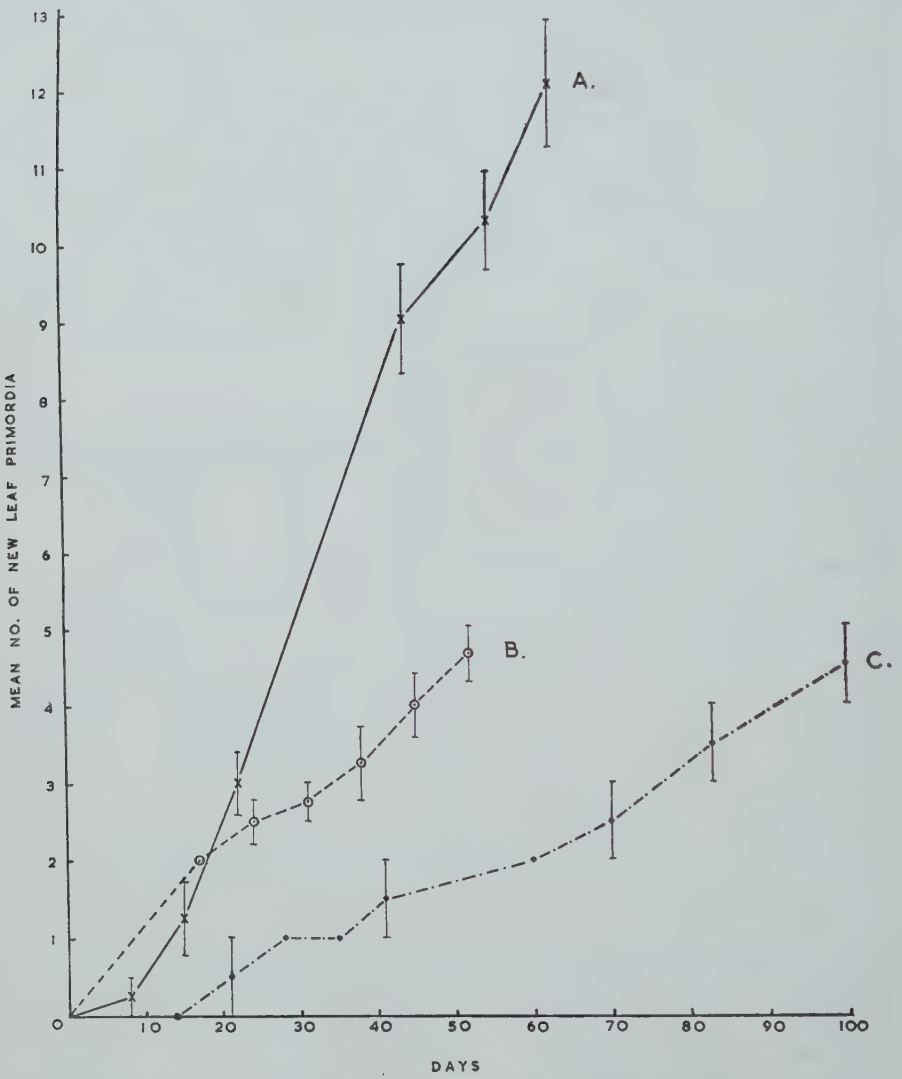


FIG. 6. Expts. 1–4. The mean rate of inception of leaf primordia, (*A*) during the first period of observation, at the beginning of the experiment, from June 30 to September 1, 1953; (*B*) during the second period, from October 7 to November 28, 1953; (*C*) during the third period, from February 27 to June 7, 1954 (expts. 2 and 4 only). The points on curves *A* and *B* represent the mean of four specimens, those on curve *C* the mean of two specimens. Vertical lines represent twice the standard error.

present case the leaf primordia which had their inception on an apex of diminished size were indeed smaller *at their inception* than those which arose on the same, larger apex of an earlier period, but there was a striking difference in their subsequent development. On the diminished apex the successive leaf primordia showed a much greater difference in size; they were also of

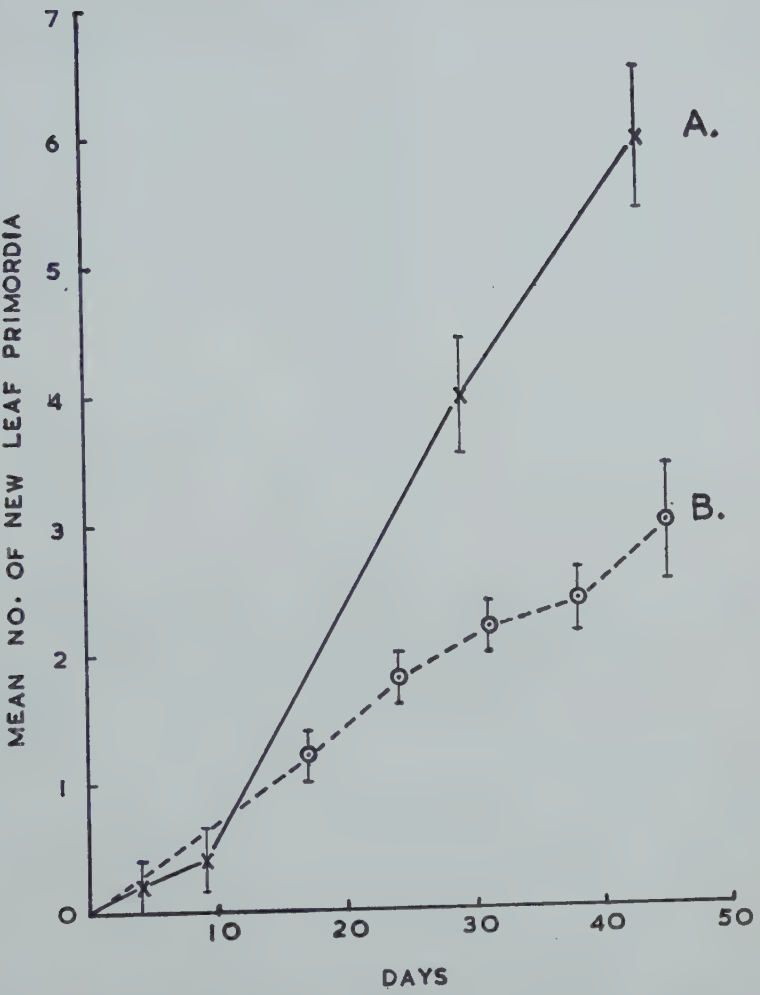


FIG. 7. Expts. 5–9. The mean rate of inception of leaf primordia, (*A*) during the first period of observation, at the beginning of the experiment, from July 14 to August 26, 1953; (*B*) during the second period of observation, from October 7 to November 21, 1953. The points represent the mean of five specimens. Vertical lines represent twice the standard error.

greater size relative to the subtending shoot apex, and they attained a greater degree of morphological complexity at an earlier actual age and plastochrone number (Table II and Figs. 1–5). Again, whereas in large, newly dissected apices from mature plants leaf primordia only begin to show circinate vernation at about the stage of P_8 and pinnation within the range of P_{20}–P_{29} (Table III), in the small apices of sporeling plants P_2 is usually circinate and may even be pinnate (Table III and Fig. 8). In apices which had been maintained under unfavourable conditions for several months, leaf primordia exhibited circinate vernation and became pinnate at a much earlier stage than is normal for mature

TABLE I

Plastochrone Duration in Apices maintained under Unfavourable Conditions

Experiment No.	Dates.	Mean plastochrone (days) over the period.
1–4 First period (A)	30/6/53 to 1/9/53	5·25
1–4 Second period (B)	7/10/53 to 28/11/53	11·16
2 and 4 Third period (C)	27/2/54 to 7/6/54	22·2
5–9 First period (A)	14/7/53 to 26/8/53	7·17
5–9 Second period (B)	7/10/53 to 21/11/53	15·0

TABLE II

Morphological Analysis of Leaf Primordia on Apices maintained under Unfavourable Conditions for Several Months

Primordium.	No. of such primordia.	Time after their inception.	Morphological condition.
P_2	1	2 weeks	Circinate
P_2	5	3 ,,	,,
P_3	2	3 ,,	,,
P_3	1	4 ,,	,,
P_3	2	4 ,,	Pinnate
P_4	2	6 ,,	,,

TABLE III

Morphological Analysis of Leaf Primordia on Apices of Mature Plants and Sporelings immediately after being laid bare

	Mature plants (collected 6/12/54).		Sporeling plants (collected 27/11/54).	
Specimens.	Youngest circinate primordium.	Youngest pinnate primordium.	Youngest circinate primordium.	Youngest pinnate primordium.
A	P_9	P_{28}	P_3	P_4
B	P_7	P_{24}	P_2	P_4
C	P_8	P_{25}	P_3	P_3
D	P_9	P_{27}	P_2	P_3
E	P_{10}	P_{29}	P_2	P_3
F	P_6	P_{21}	P_2	P_2
G	P_7	P_{20}	P_1	P_2
H	P_8	P_{27}	P_2	P_3
I	P_8	P_{22}	P_2	P_4
J	P_9	P_{26}	P_2	P_4

plants (Table II); in fact, a reversion towards the condition in sporeling plants was induced.

In starved specimens the ratio between the radial distances of two successive leaf primordia from the centre of the shoot apex, the *plastochrone ratio* (Richards, 1948, 1951), is considerably higher than at the beginning of the experiments. From such measurements Richards has calculated a value known as the *phyllotaxis index*, so that with a knowledge of the plastochrone ratio it is possible to read off values of the phyllotaxis index, and hence the phyllotactic system of the plant, from tables (Richards, 1951). In the present work the plastochrone ratios were calculated from camera lucida drawings

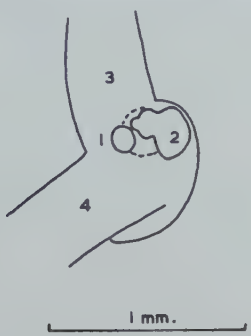
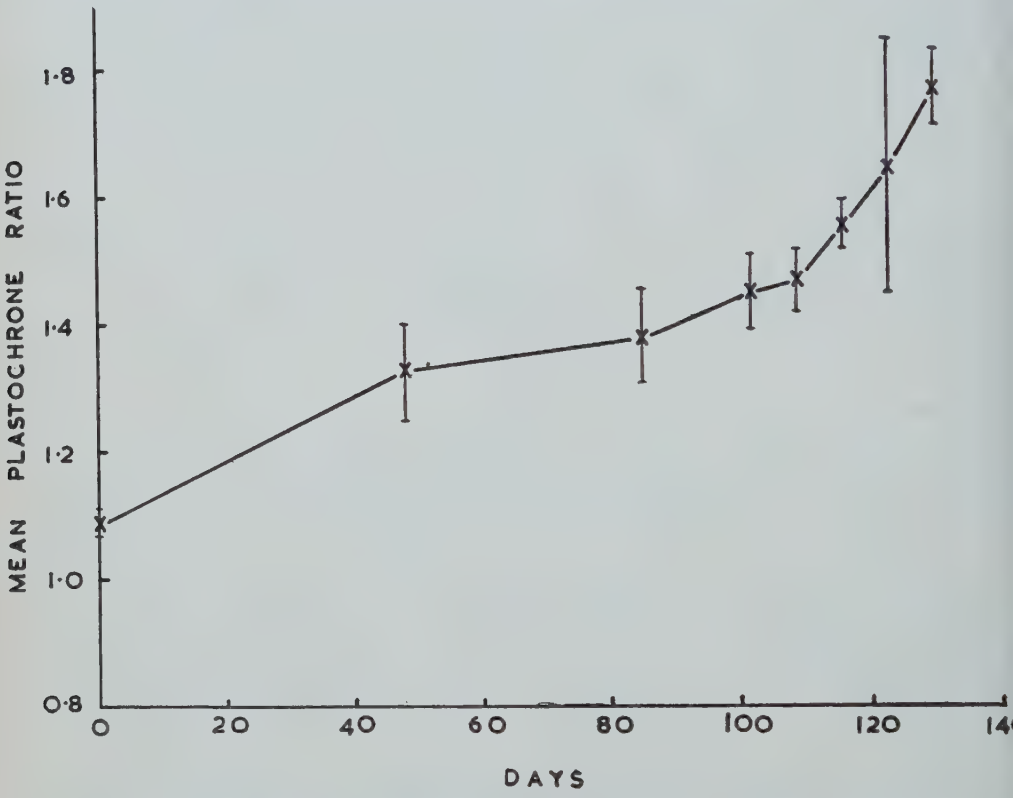


FIG. 8. The apex of a young sporeling. P_2 is becoming pinnate, and is markedly circinate, obscuring the centre of the apical cone. (×26.)

(×35) made at approximately weekly intervals. Measurements were only obtained of the radial distances from the centre of the three youngest primordia, since, when the apex became very small, all the older, circinate leaf primordia had to be removed so as to leave the apex and younger primordia unobscured. The plastochrone ratio was therefore calculated from these measurements. Richards (1951) has pointed out that such measurements are subject to considerable error, since the primordia are situated in different sectors of the apex; the slight fluctuations in the plastochrone ratio observed may have been partly due to this, as well as to errors in estimating the centre of the apex, and to any slight tilt of the specimens when drawn. The measurements were not made consistently at any particular stage of the plastochrone.

Notwithstanding occasional fluctuations in the plastochrone ratio of individual specimens, it is clear from Figs. 9–11 that there was a marked increase in plastochrone ratio, and hence a decrease in phyllotaxis index, over the period of observation. This indicates that there was a change from a higher system of phyllotaxis, characteristic of the mature plant, to a lower system, characteristic of sporelings (Table IV). A phyllotaxis index of 3·7 or 3·8 indicates a system of phyllotaxis slightly nearer to a (5+8) orthogonal system than a (3+5) system, and one of 2·0 is equivalent to a (2+3) system (Richards, 1951). De Bary (1884) states that sporelings of *Aspidium* (*Dryopteris*) *filix-mas* have a 1/3 phyllotactic system (equivalent to a (1+2) orthogonal system) while mature plants have a 5/13 or even 8/21 system (equivalent to a (5+8)

or (8+13) orthogonal system). Starvation has therefore led to a reversion of the phyllotactic system of these specimens of *Dryopteris aristata* towards the lower systems prevailing in sporeling plants. The change was evident in the radial rather than the tangential spacing of the primordia, since individual angles of divergence were rather variable. Wardlaw (1948) has already shown



FIGS. 9–10. The change in plastochrone ratio with progressive starvation of the apices. FIG. 9. Vertical lines represent twice the standard error. Expts. 5–9; the majority of points represent the mean of five specimens, the last two points being the mean of two specimens only.

that both large and small apices of *Cyathea* have a similar angle of divergence, but it is apparent from his drawings that in that fern also there is some difference in the radial spacing of primordia in large and small apices.

TABLE IV

The Plastochrone Ratio and Phyllotaxis Index of Sporeling Plants

Sporeling.	Plastochrone ratio.	Phyllotaxis index.
A	1·86	1·7
B	1·6905	2·0
C	1·563	2·1
D	1·415	2·3

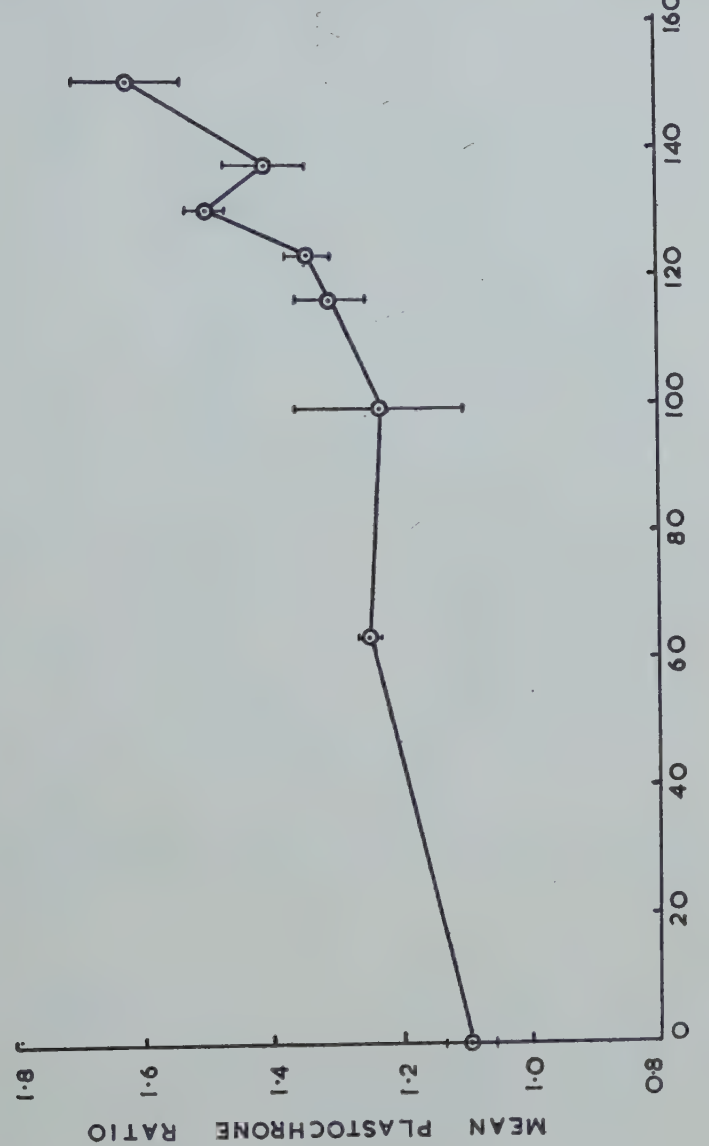


FIG. 10. Expts. 1–4; points represent the mean of from two to four specimens. Vertical lines represent twice the standard error. See Fig. 9.

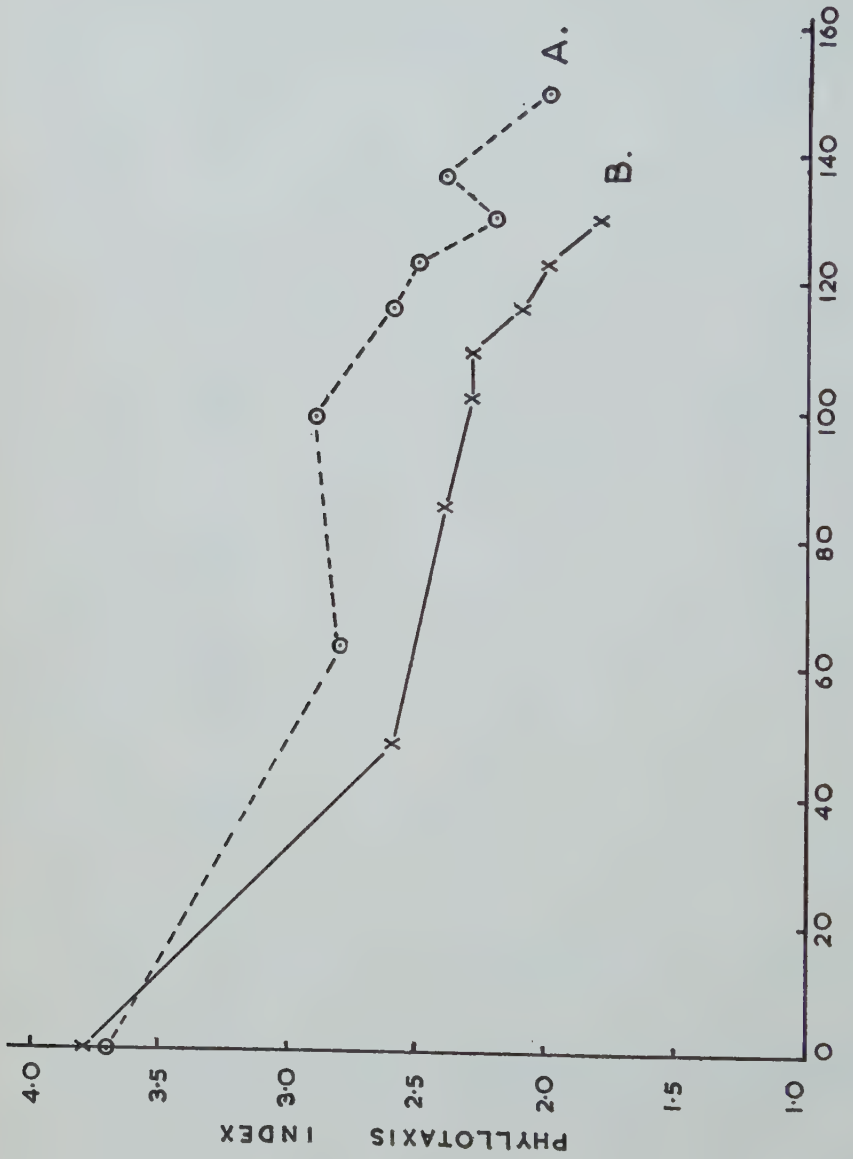


FIG. 11. The change in phyllotaxis index with progressive starvation of the apices. (*A*), expts. 1–4; (*B*), expts. 5–9.

A reversal in the direction of the genetic spiral was observed on four occasions (Fig. 12). In some cases this was due to a primordium arising almost opposite to the preceding one, because of damage to one of the flanking primordia, but this explanation was not always applicable.

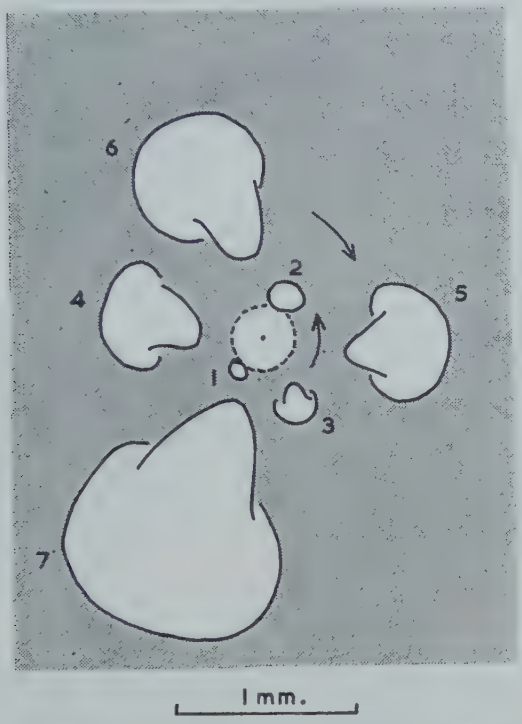


Fig. 12. Expt. 5. Surface view of the shoot apex, showing a change in the direction of the genetic spiral between P_5 and P_4. 112 days after the beginning of the experiment. (×24.)

Anatomy

The vascular structure of the attenuated specimens was dictyostelic, with a solenostele near the tip of the apex (Figs. 13–18). Short segments of the axis were sometimes solenostelic (Fig. 17), this being attributable to the longer internodes of the attenuated plants and to the destruction of young leaf primordia. Usually only three meristeles were present in a transverse section of the axis. The petioles of these specimens possessed only two small meristeles (Figs. 13–16); they consisted of fully differentiated vascular tissue. In one specimen, in which the apex eventually became parenchymatous, some of the petioles possessed only one meristele. Very young leaf primordia consisted of normal meristematic tissue and possessed normal prestelar tissue (i.e. incipient vascular tissue). Starch grains were not observed in the tissues.

FIGS. 13–18. Serial transverse sections of an attenuated apex (expt. 5). Fig. 13. A solenostele of prestelar tissue is present, near the apex. In Fig. 17 the axis is solenostelic for a short distance; in Fig. 18 it is dictyostelic again. (Vascular tissue black; prestelar tissue dotted.) (×21.)

DISCUSSION

The observations recorded here show that in *Dryopteris aristata* the nutritional status of the shoot apex is reflected in its size, in the size of its lateral organs, the rate of leaf inception and development, the system of phyllotaxis, and the vascular structure of the axis. Such a demonstration serves to emphasize the close interrelationship between these various mani-

festations of apical growth, and confirms Wardlaw's (1948) finding that, in the ferns, apical organization remains stable over a considerable size range.

Bower's (1930) view that the number of lateral organs and vascular strands of a plant is dependent upon the size and strength of the shoot apex has received general confirmation from these studies. Ball (1949) observed an increase in the number of vascular bundles in the stem of *Lupinus* as a feature of the obconical development of the plant, and Esau (1954) considers that such observations may be associated with changing phyllotaxis. It is therefore of some interest that in these attenuated apices of *Dryopteris aristata* a simplified vascular pattern in the axis was correlated with a change to a lower phyllotactic system.

Richards (1948) states that the plastochrone ratio may change as a result of alteration in the size of the apex or of the leaf primordium at its inception, or both. Except in the case of apices approaching the flowering condition, these factors vary with age, and he considers that 'much of the alteration in the ratio with age probably reflects nutritional factors'. The records and illustrations presented here (see Figs. 1–5) show that the change in phyllotaxis was due to a differential change in the sizes of the apex and the leaf primordia, the former being more drastically reduced by starvation than the latter. While cases of reduction in phyllotactic systems due to starvation have been described before (e.g. Church, 1904), a progressive reversal of the normal ontogenetic lowering of the plastochrone ratio does not appear to have been demonstrated previously.

The change in the rates of inception and development of leaf primordia is also correlated with the system of phyllotaxis. Richards (1948) points out that in low phyllotactic systems the size of the primordium relative to that of its subtending shoot apex is much greater, so that it is necessary for the apex to regenerate a much greater relative volume of tissue during the plastochrone, which must therefore be of long duration. In higher systems the plastochrone is short, and numerous primordia in various stages of inception and development are present. Thus, in large apices of *Dryopteris aristata* with higher systems of phyllotaxis, the three youngest leaf primordia may all be capable of development as shoot buds if isolated from the shoot apex by a deep tangential incision (Cutter, 1954), but this would clearly not be the case in sporelings and young plants with lower systems of phyllotaxis, where leaf development is more rapid. Isolation of I_1 and P_1 in sporelings of *D. aristata*, however, still results in the development of a bud.

That the morphological development of leaf primordia is more rapid under conditions of starvation than under more favourable nutritional conditions may be due to the fact that, because of the small size of the apex, the primordia rapidly attain the sub-apical region, where the major part of their development takes place (Wardlaw, 1952). This investigation has yielded no precise information as to which of the necessary substances limits the growth of the shoot meristem under the experimental conditions, but it seems unlikely that serious carbohydrate deficiency is involved. Richards (1948) has already

drawn attention to the lack of precise studies on the effect of various nutrients on phyllotaxis, and the present work lays further emphasis on the need for nutritional studies in relation to development.

Summary

Under the experimental conditions described, progressive starvation affects shoot apices of *Dryopteris aristata* as follows:

1. The apex decreases very markedly in size.
2. The size of leaf primordia at their inception decreases, but their size relative to the shoot apex increases.
3. The rate of inception of leaf primordia decreases.
4. The rate of morphological development of leaf primordia increases.
5. There is a reduction in the system of phyllotaxis towards that characteristic of sporelings.
6. The vascular system of the attenuated axes is dictyostelic, but there is a reduction in the number of meristeles in the axis and in the leaves.

Acknowledgements

I wish to thank Professor C. W. Wardlaw for his helpful guidance and encouragement throughout the investigation. This work was carried out during the tenure of a Berry Scholarship awarded by the University of St. Andrews, for which grateful acknowledgement is made.

Literature Cited

Abbe, E. C., and Phinney, B. O., 1951: The Growth of the Shoot Apex in Maize: External Features. Amer. J. Bot., xxxviii. 737.

Allsopp, A., 1953: Experimental and Analytical Studies of Pteridophytes. XIX. Investigations on *Marsilea*. 2. Induced Reversion to Juvenile Stages. Ann. Bot. N.S., xvii. 37.

—— 1953*a*: Experimental and Analytical Studies of Pteridophytes. XXI. Investigations on *Marsilea*. 3. The Effect of Various Sugars on Development and Morphology. Ibid., xvii. 447.

—— 1954: Juvenile Stages of Plants and the Nutritional Status of the Shoot Apex. Nature, London, clxxiii. 1032.

Ball, E., 1949: The Shoot Apex and Normal Plant of *Lupinus albus* L., Bases for Experimental Morphology. Amer. J. Bot., xxxvi. 440.

Bary, A. de, 1884: Comparative Anatomy of the Phanerogams and Ferns. Oxford.

Bower, F. O., 1930: Size and Form in Plants. London.

Church, A. H., 1904: On the Relation of Phyllotaxis to Mechanical Laws. London.

Cutter, E. G., 1954: Experimental Induction of Buds from Fern Leaf Primordia. Nature, London, clxxiii. 440.

Esau, K., 1954: Primary Vascular Differentiation in Plants. Biol. Rev., xxix. 46.

Richards, F. J., 1948: The Geometry of Phyllotaxis and its Origin. Soc. Exp. Biol. Symposia II, Growth in Relation to Differentiation and Morphogenesis. Cambridge, p. 217.

—— 1951: Phyllotaxis: its Quantitative Expression and Relation to Growth in the Apex. Phil. Trans. Roy. Soc. B, ccxxxv. 509.

Sinnott, E. W., 1921: The Relation between Body Size and Organ Size in Plants. Amer. Nat., xv. 385.

Snow, M., and Snow, R., 1931: Experiments on Phyllotaxis. I. The Effect of Isolating a Primordium. Phil. Trans. Roy. Soc. B, ccxxi. 1.

Steeves, T. A., and Wetmore, R. H., 1953: Morphogenetic Studies on *Osmunda cinnamomea* L.: Some Aspects of the General Morphology. Phytomorphology, iii. 339.

Wardlaw, C. W., 1944: Experimental and Analytical Studies of Pteridophytes. IV. Stelar Morphology: Experimental Observations on the Relation between Leaf Development and Stelar Morphology in Species of *Dryopteris* and *Onoclea*. Ann. Bot., n.s., viii. 387.

—— 1945: Experimental and Analytical Studies of Pteridophytes. VI. Stelar Morphology: The Occurrence of Reduced and Discontinuous Vascular Systems in the Rhizome of *Onoclea sensibilis*. Ibid., ix. 383.

—— 1948: Experimental and Analytical Studies of Pteridophytes. XIII. On the Shoot Apex in a Tree Fern, *Cyathea Manniana* Hooker. Ibid., xii. 371.

—— 1949: Further Experimental Investigations of the Shoot Apex of *Dryopteris aristata* Druce. Phil. Trans. Roy. Soc. B, ccxxxiii. 415.

—— 1952: Experimental and Analytical Studies of Pteridophytes. XVIII. The Nutritional Status of the Apex and Morphogenesis. Ann. Bot., n.s., xvi. 207.

Whaley, W. G., 1939: Developmental Changes in Apical Meristems. Proc. Nat. Acad. Sci., xxv. 445.

The Development and Structure of the Root-nodules of *Myrica gale* L. with Special Reference to the Nature of the Endophyte

BY

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With Plates XVIII and XIX and three Figures in the Text

Abstract

The development and structure of the root nodules of *Myrica gale* are described, and evidence advanced showing that they represent modified lateral roots. Attempts at the isolation of the endophyte were unsuccessful, but on the basis of a cytological study of the nodules the author adheres to the view of some previous investigators that the endophyte is actinomycetal.

Introduction

THE root nodules regularly present on *Myrica gale* L. ('Bog Myrtle' or 'Sweet Gale') were first reported and described by Brunchorst (1886–7), and although they have received investigation by a number of subsequent workers, our knowledge concerning the nodules is still very incomplete. The method of infection of the root by the nodular endophyte and the subsequent stages of early nodule development have remained uninvestigated, while there has been much diversity of opinion as to the identity of the endophyte. The literature on this last aspect was reviewed recently by Hawker and Fraymouth (1951). Different authors have variously classified the endophyte as fungal, bacterial, actinomycetal, or plasmodial in nature, this uncertainty being largely due to the difficulty that it has so far proved impossible to isolate the organism. Three authors (Peklo, 1910; Bottomley, 1912; and Youngken, 1919) have claimed to have effected isolation, but, as will be shown later, their proof of this is unsatisfactory.

Interest in these nodules has been increased by the demonstration that fixation of atmospheric nitrogen is associated with them, and that in other ways a close resemblance to leguminous nodules is shown (Bond, 1951; Bond, Fletcher, and Ferguson, 1954).

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]

Methods

It appears that all previous investigators of structural and developmental aspects of *Myrica* nodules have relied upon field material. Using the methods described by Bond (1951), the present author has raised plants from seed in the greenhouse, for the most part in water culture, and the availability of this material has greatly facilitated the study of nodule development. The procedure has been to germinate the seed (after cold-treatment) in peat, and then to transplant the seedlings at the two-leaf stage into Crone's solution (nitrogen-free formula, adjusted to pH 5·4). The seedlings were then inoculated by applying to the roots a suspension of crushed nodules from other *Myrica* plants, the usual proportion being approximately 3·5 g. nodules to 100 ml. water. Without this inoculation the plants do not develop nodules under the conditions of culture employed.

Material for anatomical study was fixed in Bouin's fluid. Microtome sections were cut 6–7μ in thickness, and were stained with safranin and fast green, or, for cytological examination, with the tannic acid, iron alum, safranin, and orange G combination of Sharman (1943). The distribution of suberised layers was determined by the use of Sudan III, lignified walls by means of phloroglucinol and hydrochloric acid, and tannin by the nitrous ether method of Vinson (1910).

Observations

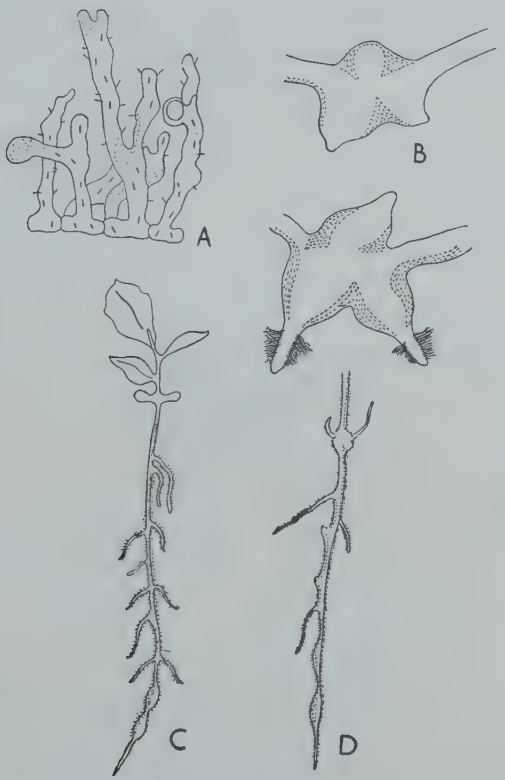
A. *Some early effects of inoculation*

The roots existing at the time of transplanting, and those which develop soon afterwards, are richly clothed with root-hairs. When roots were examined microscopically 4 or 5 days after inoculation, it was seen that the root-hairs had become twisted and branched, effects which became more marked as time went on. The root-hairs of uninoculated plants remained straight and unbranched. These differences in the condition of the root-hairs resulted in effects which were apparent to the naked eye (Pl. XVIII, Fig. 1).

Following on this contortion, many of the root-hairs, and also cells of the piliferous layer, became filled with granular material which gave a tannin reaction. Sometimes the root-hairs had many rod-shaped bacteria-like structures attached end-on to them, and in a few cases similar structures were detected within the root-hairs (Text-fig. 1 A). Direct microscopic examination of crushed nodule inoculum as used for the inoculation of plants (p. 502) showed the presence, after 24 hours' incubation, of similar rod-shaped organisms around and within fragments of nodule tissue. No infection threads were observed in the contorted root-hairs, the examination of which, however, was made difficult by the presence of the tannin already noted.

Concurrently with or slightly later than the events taking place in the root-hairs the cortical cells of the root (as seen in sections) became filled with granular tannin and in the trabecular type of cortex (see later) the filaments of

cells became twisted and contorted in a manner reminiscent of infected root-hairs. These developments presumably indicate the arrival of the endophyte. They are not confined to one particular side of a root. Thus all the trabeculae in any one section were often filled with tannin, suggesting that there had been mass infection.



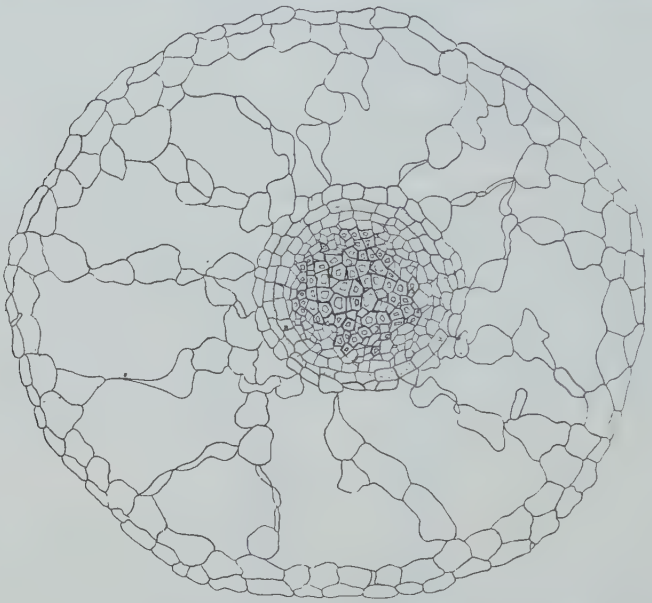
TEXT-FIG. 1. A. Contorted root-hairs of *M. gale* showing presence of bacteria-like bodies around and within them. Tannin is denoted by dots. (×250.) B. Two stages in the development of nodules. Upper: triple nodule 3 weeks after inoculation. Lower: same nodule, 2 weeks later. Note nodule-roots bearing root-hairs. This is not a common feature of nodule-roots. (×15.) C. Young plants of *M. gale* (12 weeks old) showing early development of nodules 2 weeks after inoculation. (×1.) D. Roots of *M. gale* showing stages in nodule formation. Note upwardly growing nodule-roots. (×1.)

B. *External features of nodule development*

Nodules visible to the naked eye are often present 2 weeks after inoculation, and at this stage appear as lateral swellings on the parent root (Text-fig. 1 C). Microscopically the nodules can be detected several days earlier. Their emergence from the parent root causes more disruption of the tissues than in the case of an ordinary lateral root, leading occasionally to a splitting of the parent root. Very often two or three nodules arise near together (Text-fig. 1 B). The nodule is at first a globular structure, but soon becomes pear-shaped, and from its distal pointed end a fine root, termed by Bond (1951) a

nodule-root, grows out, in some cases 2–3 weeks after the first appearance of the nodule. As was first observed by Bond (loc. cit.) these nodule-roots grow upwards (Text-fig. 1 D). They are typically unbranched and devoid of root-hairs, though exceptions occur (Text-fig. 1 B). A nodule-root has never been observed to give rise to a fresh nodule.

Near the junction of the nodule-root and the nodule from which it arises three new nodule lobes are formed from each of which a nodule-root in due



TEXT-FIG. 2. T.S. Root of *Myrica gale* from uninoculated plant grown in water culture with added nitrate. (×175.)

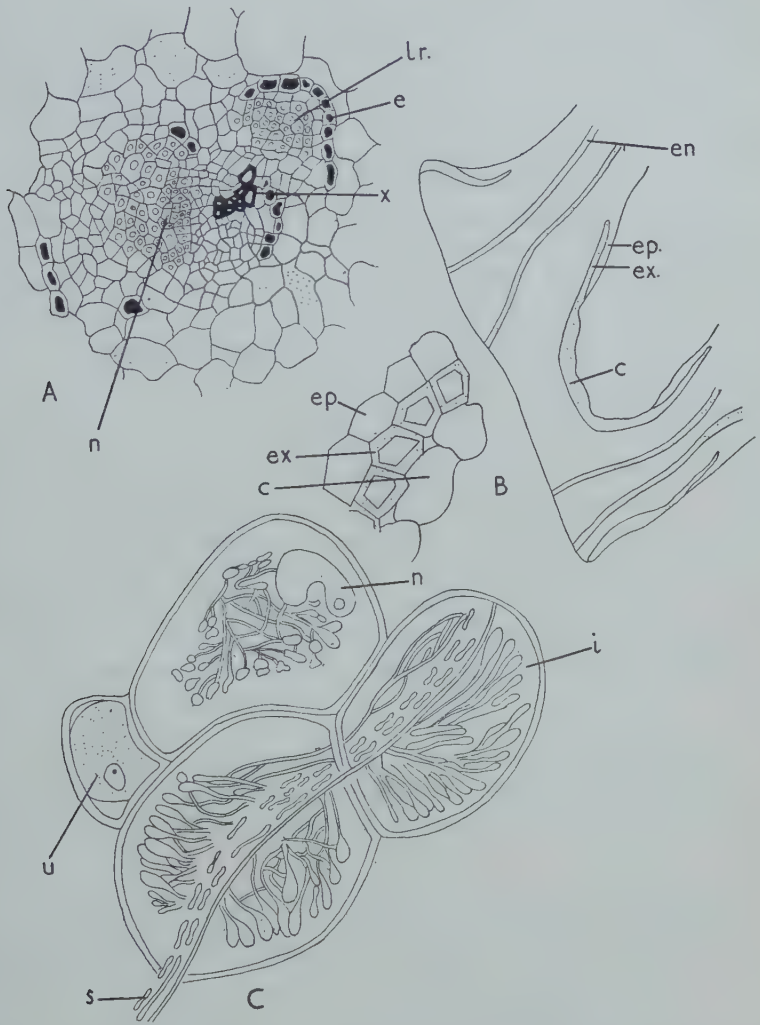
course grows. This process continues, giving rise eventually to a close cluster of nodule lobes. The nodules themselves are often completely hidden by the upward-growing nodule-roots (Pl. XVIII, Fig. 2). Young nodules frequently have an intense red coloration, shown by Bond (1951) to be due to anthocyanin. Later this colour is replaced by the characteristic light brown tint of the mature nodule.

The above remarks apply to nodules on plants in water culture. Development in the field proceeds along similar lines. In the early summer, field nodules bear newly-formed and prominent white nodule-roots which attain a maximum length of 4–5 cm. (Pl. XVIII, Fig. 3). Later these roots wither, and when examined in winter or spring appear as wispy brown threads (Pl. XVIII, Fig. 4).

C. *Internal features of nodule development and structure*

The root of an uninoculated plant grown in water culture containing an inorganic nitrogen source shows, as seen in transverse section (Text-fig. 2), a

cortex which in the young condition consists of parenchymatous cells with small air-spaces, but which later develops a trabeculate structure. The xylem is inconspicuously tetra- or pentarch.



TEXT-FIG. 3. A. Part of T.S. of root of *M. gale* showing lateral root (*lr*) and nodule (*n*) forming. Note also endodermis (*e*) and xylem of parent root (*x*). (×360.) B. Upper: longitudinal section of apical part of nodule with bases of two nodule-roots showing distribution of suberised tissues (dotted). (×75.) Lower: outer tissues of base of nodule-root showing suberised exodermis (×200.) *ep*, epidermis; *ex*, exodermis; *en*, endodermis; *c*, cortex. C. Nodule cells of *M. gale*. Note large infected cells (*i*), one having a lobed nucleus (*n*). Many of the hyphae end in clubs. Note also segmented hyphae (*s*). Uninfected cell (*u*). (×1,350.)

The earliest stage in the initiation of a nodule that has been found in microtome sections of inoculated roots is that shown in Pl. XVIII, Fig. 5. The young nodule is seen as a somewhat spherical mass of meristematic cells which

is enclosed by the parent-root endodermis and has obviously arisen from the pericycle. A similar stage is shown in Text-fig. 3 A, a root initial being in addition present here. The young nodule can be distinguished from an ordinary lateral root initial by differences in shape, the root being conical rather than spherical, and it emerges from the parent root with much less disturbance of the surrounding tissues. The cells of the young nodule are mostly filled with tannin which is absent from the cells of the young root. The presence of this tannin makes it almost impossible to decide whether or not the endophyte is present in recognizable form in the cells.

In the early stages of development the nodule has no clear plerome or vascular strand, and neither has it a protective cap. Eventually an apical meristematic zone is established and a centrally-placed vascular strand is differentiated which links up with the corresponding tissues of the parent root (Pl. XVIII, Fig. 6). The apical meristematic cells are small and densely filled with tannin, so that here again detection of the endophyte is difficult. Farther back towards the base of the nodule, however, the cells become larger and closely coiled masses of the fine hyphae of the endophyte can be seen within some of them. These cells have lost their tannin contents. They are enlarged and distributed without order through the cortical region of the nodule. The remaining cells of the cortex are filled either with tannin or starch grains.

Soon after emergence of the nodule from the parent root a cork cambium is established in the outer cortex of the nodule and this cuts off a shallow layer of cork cells to the exterior. Concurrently with this the nodule-root grows out from the tip of the nodule. In the nodule-root the cork layer is replaced by a suberised exodermis which, however, extends only a short way into the nodule-root. (Text-fig. 3 B). The root has a prominent root-cap and shows the same general structure as a normal root.

A transverse section of a nodule which has passed through the developmental stages so far described appears as in Pl. XIX, Fig. 1. The structure of a nodule in this condition was first described by Chevalier (1900–2). The main features are as follows:

1. An outermost covering of 2–4 layers of narrow suberised cork cells containing a substance giving the tannin reaction. Chevalier describes this as 'gummy lignin' or 'wound gum' and lists a series of reactions by which it may be recognized.
2. A hypertrophied cortex comprising larger infected cells and smaller uninfected ones. The former are 2–3 times as large as the uninfected cells. Their walls have been reported by previous authors to be lignified or suberised. The present author's finding is that reactions for both substances are given. The uninfected cells frequently contain starch grains or tannin.
3. An endodermis, with its cells in the secondary condition and densely filled with tannin. The endophyte does not penetrate into or beyond the endodermis, which appears to form a 'tannin barrier' similar to that noted by Clowes (1951) in the mycorrhizal roots of *Fagus sylvatica*.

4. The stele, with 3–4 layers of irregularly-shaped pericyclic cells, poorly developed phloem, and typically tetrarch xylem.

In a longitudinal section of a slightly older nodule the production of new nodule lobes can be seen to be occurring towards the apex, with the meristematic apices showing dense tannin-filled cells (Pl. XIX, Fig. 2).

D. *Cytology of the nodules*

Microtome sections, stained as already described, show that the infected cells of the nodule cortex are almost filled by a dense network of what the author has no hesitation in describing as hyphae (Pl. XIX, fig. 3). These are less than 1μ in diameter and often show a radiating arrangement in the cells, the ends of the hyphae being frequently club-shaped (Text-fig. 3 c). Single hyphae or strands of hyphae can be seen passing from cell to cell. No, or only very occasional, septa can be seen in most of the hyphae. Some, however, have segmented, giving the appearance of bacteria-like bodies lying end to end. It is possible that these are of the same nature as the bodies noted (p. 503) around and within the root-hairs and that they are responsible for initiation of nodule formation. Hand sections mounted in water, from fresh nodules, show equally convincing hyphae. The nucleus of the infected cell can clearly be seen; it frequently doubles in size and becomes lobed.

In older nodule cells the appearance of the endophyte is somewhat different. The contents of the hyphae have disappeared, and the width of the hyphae has increased to some 2–2·5μ. The empty hyphae are distorted and twisted (Pl. XIX, Fig. 4). Often in such cells the nucleus has disappeared.

In what appears to be a still later stage the empty hyphae form a dark structureless mass reminiscent of that seen in the digestive cells of the root of *Neottia* (Pl. XIX, Figs. 5 and 6).

E. *Attempted isolation of the endophyte*

In this investigation the methods employed by Peklo (loc. cit.), Bottomley (loc. cit.), and Youngken (loc. cit.) were repeated as closely as possible. In most cases the media remained sterile, but occasionally organisms were observed and any isolate, whether fungal, bacterial, or actinomycetal, was employed in reinoculation tests with plants of *Myrica gale*. All failed to induce nodulation and were presumably contaminants. Further attempts at isolation using other sterilization techniques, media, and conditions (including anaerobic conditions) similarly failed to produce nodule-forming organisms.

Discussion

1. *Method of infection*

The most positive feature observed following inoculation of *M. gale* plants is the deformation of the root-hairs. This feature has been observed also in other non-leguminous nodule-forming plants, e.g. in *Alnus* (Hiltner, 1903). Root-hair curling is of course considered to be a pre-requisite for infection of legumes by *Rhizobium* (Thornton, 1952).

It seems almost certain that infection in *Myrica* must occur through the root-hairs, the only other possibility being infection *via* breaks in the cortex where lateral roots emerge, as determined by Allen and Allen (1940) for peanut. In *M. gale*, however, nodules have been found far removed from the site of lateral roots and this possibility is therefore discounted. Since the present investigation was not carried out using a pure culture of the endophyte, it cannot be stated with certainty that the bacteria-like bodies observed within the root-hairs are the infecting organism, but the failure to observe any other organism and the presence of such bodies within the nodule points strongly to this being the case.

2. *Morphological nature of the nodule*

The nodule, arising in the pericycle, is evidently a modified root, the full development of which is for a time arrested by the nodule-forming process. It is continued as the nodule-root. That the nodule-root is a true root is shown by the presence of a root-cap and by the fact that it occasionally bears root-hairs.

3. *The nature of the endophyte*

Despite many attempts, using a variety of media at various pH's and temperatures, the present author has been unable to isolate the endophyte in pure culture. A similar failure has been reported by Arcularius (1928) and Hawker and Fraymouth (1951). As indicated already, three authors, namely Peklo (1910), Bottomley (1912), and Youngken (1919), have claimed success and have based the identification of the endophyte on their isolations. Peklo (loc. cit.) described it as an actinomycete consisting of slimy zoogloeal threads, Bottomley (loc. cit.) as an organism similar to *B. radicicola*, and Youngken (loc. cit.) as an actinomycete consisting of non-septate thin filaments, rods, and coccus forms which he named *Actinomyces myricarum*. Close examination of their papers reveals that none of them achieved satisfactory nodulation with their supposed isolates. Owing to lack of seedlings Peklo performed no re-inoculation tests. Bottomley watered two plants with a suspension of the isolated organism and observed that at a later stage the plants began to grow vigorously whereas previously growth was poor, but it is not stated in the body of the paper whether this was accompanied by nodule formation (though there is a statement to this effect in the summary). If nodulation did occur, then it should not necessarily be ascribed to the presence of the cultured organism since earlier some of the uninoculated controls also developed nodules and had to be discarded. Youngken removed five seedlings of *M. cerifera* from the soil, surface-sterilized their roots, and then small portions of the actinomycete culture were pricked into the roots of four seedlings, a control being pricked with a sterile needle only. They were planted in sterile sand and watered with sterile Knops solution. After 9 weeks the plants were examined for tubercles, and according to Youngken 'these were found in a primitive state at the point of inoculation on all but two including the control'

(i.e. on three). 'Thin hand sections revealed the presence of actinomyces in the same condition as observed in cells of tubercles of *M. cerifera*.' It seems to the present author that the finding of a few hyphae within cortical cells cannot be accepted as nodule formation. Using crushed nodule suspension for inoculation, nodules have been observed with the naked eye on *M. gale* plants some 14 days after inoculation, as already noted. If Youngken's isolate was the endophyte, then by the end of 9 weeks it ought to have formed well-marked nodules. He does not state whether proper nodules developed subsequently and we must assume that they did not.

The failure to isolate the causative organism from the nodule of *Myrica* is disappointing but not wholly unexpected since the endophyte of other non-legume nodule-forming plants such as *Alnus*, *Hippophaë*, and *Elaeagnus* have not yet been grown apart from the host plant.

Is, then, the relationship to be regarded as being of an obligate nature? The presence of nodules and consequently of the endophyte is not necessary for the healthy growth of the *Myrica* plant. Supplied with an inorganic source of nitrogen such as sodium nitrate or ammonium nitrate, uninoculated and non-nodulated plants of *Myrica* will grow excellently and produce viable seed; it has not yet been shown that the endophyte can survive apart from the plant, but it should not be assumed from this that it cannot be cultivated. It may be that the organism is very exacting in its food requirements and we have not yet hit upon the correct medium, or perhaps the pH tolerance is narrow. At first sight the latter does not appear to be the case, since Bond (1951) has shown that nodulation can occur over a fairly wide range (3·3–6·3). But it should be remembered that this merely indicates that the organism can survive for a time in media of these pH's, not that it can multiply in them, for the present author has determined that the pH of the *Myrica* roots remained constant between 5·0–5·3 though growing in water culture solutions of pH 4·2, 5·4, 6·3, and 7·0.

Possibly also the organism is micro-aerophilic. One attempt has been made without success to isolate it under such conditions, but many more attempts must be made before the possibility is discarded. *Myrica gale* is usually found growing in wet boggy situations where one could reasonably assume that there was an oxygen shortage and the cork layer formed around the nodule would cut down the amount of oxygen available to the organism within it. It has already been noted, too (p. 504), that the endophyte is never found in the nodule-roots. If, as Bond (1952) believes, these nodule-roots are aerating organs, it may well be that the organism is prevented from migrating to them because of the relatively high concentration of oxygen present there.

Lacking then a pure culture, identification must rest upon cytological evidence. It seems to the present author that the identification must not be carried too far on this basis and a tentative attempt is made to determine whether it be bacterium, actinomycete, myxomycete, or fungus without attempting to assign it to a particular genus or species.

The earliest workers, namely, Brunchorst (1886–7), Moeller (1890),

Chevalier (1900–2), and Harshberger (1903), believed that it was a fungus. In the case of the first three the only possible explanation is that they examined mature material in which the hyphae were empty and swollen, thus simulating fungal hyphae. They did not observe the fine threads seen by later workers. Harshberger (loc. cit.) used dried museum material which he first boiled and then treated with 35 per cent. alcohol. Obviously observations on such material can have little value.

Bottomley (1912) and Dangeard and Trnka (1929) considered that the endophyte was a bacterium. In the case of the first author the fact that he claimed to have isolated the organism as a bacterium would undoubtedly sway his judgement. Shibata and Tahara (1917), remarking on Bottomley's findings, stated that the 'bacteria' seen in the nodules were probably artefacts due to faulty technique. This is probably a reasonable explanation. Dangeard and Trnka (loc. cit.) regarded the endophyte as a filamentous bacterium which they named *Rhizobacterium myricae*. The present author has noted that in some cases the hyphae may form bacteria-like bodies, but these are derived from hyphae which in the young condition show no septa.

In a more recent paper Hawker and Fraymouth (1951) state that the endophyte in *Myrica* is a member of the Plasmodiophorales, since when modern fixatives are used the 'hyphae' have no cross-walls and no 'obvious containing walls' while in fresh material the strands are even less like hyphae. Presumably they imply that with older and less perfect fixatives, apparent containing walls might arise as artefacts. They note that the width and branching of the strands are very variable. They describe and figure club-shaped bodies which they regard as sporangia which break up into packets soon after they are formed. The particles formed from these packets have been observed by these authors to move with a dancing movement. The authors conclude, however, by saying that the *Myrica* endophyte 'is in many ways an approach to the actinomycetes'.

The present author has seen no evidence in his preparations that the endophyte is a member of the Plasmodiophorales. The virtual absence of cross-walls does not count against the organism being an actinomycete, since most investigators deny the existence of septa in actinomycetes (Henrici, 1948). As noted, the present author has examined fresh material of *M. gale* nodules both from water culture and from the field and has observed what are undoubtedly actinomycete hyphae. Since even in this fresh condition they have obvious containing walls, the latter cannot be dismissed as artefacts due to faulty fixation.

The variability in width and branching of the hyphae is not an uncommon feature of endotrophic organisms, e.g. it is observed in the hyphae of the fungus causing endotrophic mycorrhiza of orchids and there is great variability in the width of the rhizobia within any one legume nodule. It should not then be regarded as characteristic of the Plasmodiophorales. The club-like endings observed by Hawker and Fraymouth have been noticed by the present author, but he has been unable to make out any detail of their internal struc-

ture or fate. Club-formation is a very characteristic feature of many of the actinomycetes (Frobisher, 1944). According to Browning and Mackie (1949) clubs in actinomycetes are elongated pear-shaped bodies which are often seen at the periphery of the colony and have often been regarded as enlargements of the sheath around the free extremity of a filament. They are usually homogeneous and structureless in appearance and are often comparatively fragile structures which are easily broken down and may sometimes be dissolved in water.

The appearance of clubs then within the infected cells of *Myrica gale* would seem to strengthen the view that the endophyte is an actinomycete. This finding of the present author regarding the nature of the endophyte is in agreement with the findings of Shibata (1902), Arzberger (1910), Peklo (1910), Youngken (1919), and Schaede (1938–9).

Regarding the sequence of events within the infected cells, namely that the hyphae are at first thin, then widen as their contents are digested, and finally form a dark structureless clump, Schaede (1938–9) noted thick and thin hyphae within the nodule but was unable to determine whether one developed from the other or whether there were two organisms present. If two organisms are present, then both must be capable of causing nodule formation in *Myrica gale*, since both types of hyphae cause enlargement of the cells in which they are found. Furthermore the thick hyphae always appear to be devoid of contents and are probably dead. The fact too that only thin hyphae are present in the young nodule points to the thin preceding the thick in time, as does the finding of the thick hyphae at the base of the mature nodule and the thin hyphae nearer the apex.

Summary

Plants grown in water culture have been utilized in a study of the development and structure of the root-nodules of *Myrica gale*. After inoculation with crushed *Myrica* nodules the root-hairs of inoculated plants become twisted and contorted, and it is thought that the organism responsible enters the plant via the root-hairs as bacteria-like bodies which are portions of actinomycete threads.

The external and internal features of nodule formation are described. The nodule arises in the pericycle, is at first spherical but gradually becomes pear-shaped, and from its distal end a nodule-root grows out and upward. The latter seldom bears root-hairs but has a true root-cap. A central vascular strand connects the nodule to the vascular system of the parent root. The cortical region of the nodule is hypertrophied. The nodule is enclosed in a cork layer. From its origin and anatomical features it is concluded that the nodule is a modified lateral root.

It is noted that three previous authors claim to have isolated the endophyte in pure culture, but it is concluded that there is no satisfactory evidence of this. On the basis of cytological evidence it is concluded that the endophyte

is an actinomycete, thus agreeing with the findings of the majority of workers who have examined the nodules of *M. gale*. The findings of other authors that the endophyte is a fungus, bacterium, or member of the Plasmodiophorales are critically examined.

Stages in the digestion of the contents of the hyphae of the actinomycete are described and figured.

The author is indebted to Dr. G. Bond who suggested the work and has offered helpful criticism during its course, and who gave permission to publish Figs. 2, 3, and 5 of Pl. XVIII; also to Mr. W. Anderson and Mr. J. C. Raymond who assisted with photography.

Literature Cited

Allen, O. N., and Allen, E. K., 1940: Response of the Peanut Plant to Inoculation with Rhizobia, with special reference to Morphological Development of the Nodules. Bot. Gaz., cii. 121–42.

Arcularius, J. J., 1928: Zytologische Untersuchungen an einigen endotrophen Mykorrhizen. Zbl. Bakt., Abt. II, lxxiv. 191–207.

Arzberger, E. G., 1910: The Fungus Root Tubercles of *Ceanothus americanus*, *Elaeagnus argentea* and *Myrica cerifera*. 21st Rep. Mo. Bot. Gdn. 60–102.

Bond, G., 1951: The Fixation of Nitrogen associated with the Root Nodules of *Myrica gale* L. with special reference to its pH Relation and Ecological Significance. Ann. Bot., Lond., n.s. xv. 447–59.

—— 1952: Some Features of Root Growth in Nodulated Plants of *Myrica gale*. Ann. Bot., Lond., n.s. xvi. 467–75.

—— Fletcher, W. W., and Ferguson, T. P., 1954: The Development and Function of the Root Nodules of *Alnus*, *Myrica*, and *Hippophaë*. Plant and Soil, v. 4. 309–23.

Bottomley, W. B., 1912: The Root Nodules of *Myrica gale*. Ann. Bot., Lond., xxxvi (1). 111–17.

Browning, C. H., and Mackie, T. J., 1949: Textbook of Bacteriology. Oxford University Press, London.

Brunchorst, J., 1886–7: Die Structur der Inhaltskörper in den Zellen einiger Wurzelanschwellungen. Bergens Mus. Aarsber., 235–46.

Chevalier, A., 1900–2: Monographie des Myricacées — Anatomie et Histologie, Organographie. Classification et description des espèces, distribution géographique. Mém. Soc. Nation. Sci. Nat. et Math., Cherbourg, xxxii (Quatrième Série, tome ii), 85–340.

Clowes, F. A. L., 1951: The structure of Mycorrhizal Roots of *Fagus sylvatica*. New Phyt. l. 1–16.

Dangeard, P. A., et Trnka, M. L., 1929: Sur les phénomènes de symbiose chez le *Myrica gale*. C.R. Acad. Sci. Paris, clxxxviii. 1584–8.

Frobisher, M., 1944: Fundamentals of Bacteriology (3rd edn.). W. B. Saunders Company, Philadelphia.

Harshberger, J. W., 1903: The Form and Structure of the Mycodomatia of *Myrica cerifera* L. Proc. Acad. nat. Sci. Philad., lv. 352–61.

Hawker, L. E., and Fraymouth, J., 1951: A Re-investigation of the Root Nodules of Species of *Elaeagnus*, *Hippophaë*, *Alnus* and *Myrica* with Special Reference to the Morphology and Life Histories of the Causative Organisms. J. gen. Microbiol. v. 369–86.

Henrici, A. T., 1948: Moulds, Yeasts and Actinomycetes. 2nd ed. by Skinner, C. E., Emmons, C. W., and Tsuchiya, H. M. Chapman and Hall, Ltd.

Hiltner, L., 1903: Beiträge zur Mykorhizafrage. Über die biologische und physiologische Bedeutung der endotrophen Mykorhiza. Naturw. Z. Land- u. Forstw., xvii. 9–25.

Moeller, H., 1890: Beitrag zur Kenntnis der *Frankia subtilis*, Brunchorst. Ber. dtsch. bot. Ges., viii. 215–24.

Peklo, J., 1910: Die pflanzlichen Aktinomykosen. Zbl. Bakt., Abt. II, xxvii. 451–579.
Schaede, R., 1938–9: Die Actinomyceten-Symbiose von *Myrica gale*. Planta, xxix. 32–46.
Sharman, B. C., 1943: Tannic Acid and Iron Alum with Safranin and Orange G in Studies of the Shoot Apex. Stain Tech., xviii, No. 3.
Shibata, K., 1902: Die Wurzelanschwellungen von *Alnus* und *Myrica* in cytologische Studien über die endotrophen Mykorrhizen. Jb. wiss. Bot., xxxviii. 668–70.
—— und Tahara, M., 1917: Studien über die Wurzelknollchen. Bot. Mag. Tokyo, xxxi. 157–82.
Thornton, H. G., and others, 1952: A Discussion on Symbiosis involving Micro-organisms. Proc. Roy. Soc., B, cxxxix. 170–207.
Vinson, A. E., 1910: Fixing and Staining Tannin in plant tissues with nitrous ethers. Bot. Gaz., xlix. 222–4.
Youngken, H. W., 1919. The Comparative Morphology, Taxonomy and Distribution of the Myricaceae of the Eastern United States. Contr. bot. Lab. Univ. Pa., iv. 339–400.

EXPLANATION OF PLATES

Illustrating W. W. Fletcher's article on 'The Development and Structure of the Root Nodules of *Myrica gale* with Special Reference to the Nature of the Endophyte.'

PLATE XVIII

Fig. 1. Plants growing in Crone's solution in specimen tubes. Centre plant uninoculated. Left- and right-hand plants inoculated 3 weeks previously. (×⅔.)

Fig. 2. Plant in second year of growth in N-free Crone's solution. Note numerous nodules with prominent, upwardly growing nodule-roots. (×⅓.)

Fig. 3. Field nodules, collected in early summer, showing prominent white nodule-roots. (×1.)

Fig. 4. Field nodules, collected in winter, showing nodule-roots as wispy brown threads. (×1.)

Fig. 5. T.S. root with enclosed young nodule. Note that nodule cells are filled with tannin. (×250.)

Fig. 6. Young nodule emerging from the parent root, the latter seen in transverse section, 4 weeks after inoculation. (×200.)

PLATE XIX

Fig. 1. Transverse section of a nodule. Note enlarged infected cells in the cortical region. (×60.)

Fig. 2. L.S. of lobed nodule showing additional lobes forming; also nodule roots. Note nfected cells in basal region of nodule and densely filled tannin cells at apex of new lobes. (×40.)

Fig. 3. Nodule cells showing the endophyte. The smaller uninfected cells contain tannin. (×1,500.)

Fig. 4. Nodule cells showing the swollen empty hyphae of the endophyte. (×1,500.)

Fig. 5. Nodule cells in which the hyphae are beginning to form clumps due, apparently, to partial digestion. (×1,500.)

Fig. 6. Nodule cells in which the hyphae are apparently digested leaving dark staining clumps. (×1,500.)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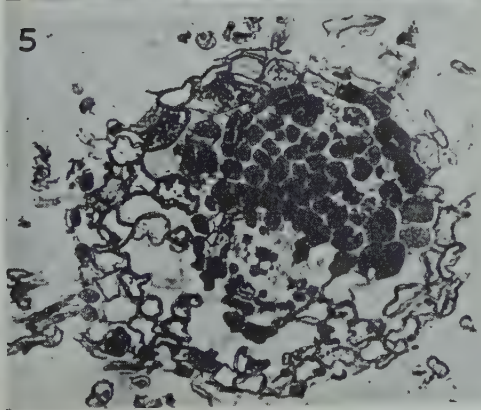
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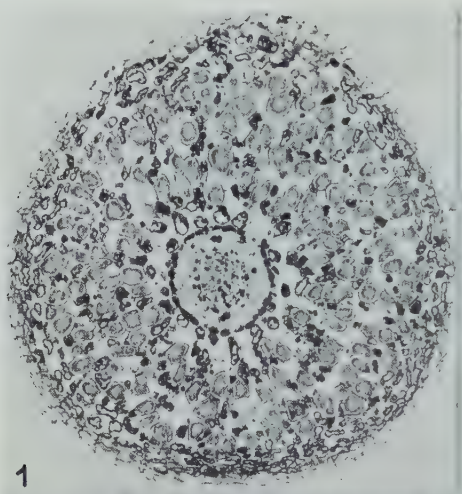
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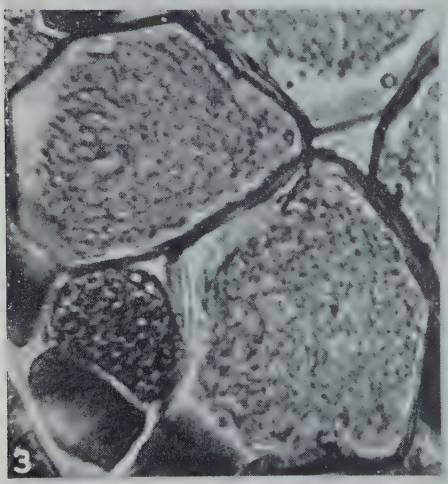
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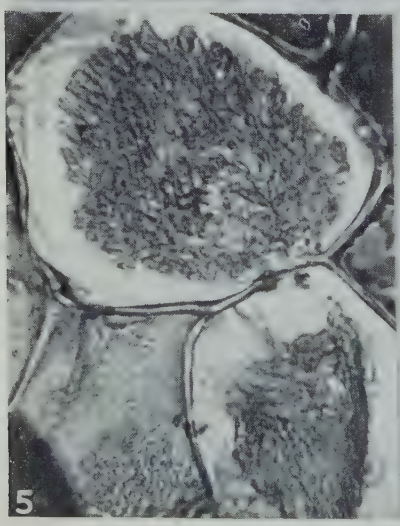
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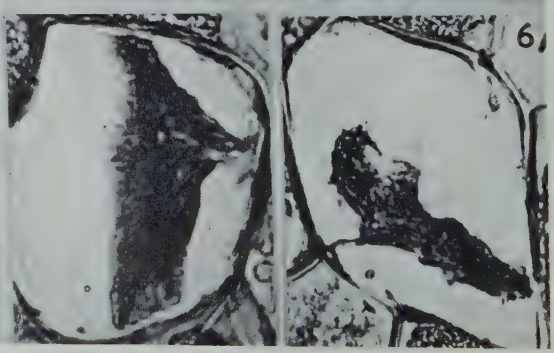
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Experimental and Analytical Studies of Pteridophytes

XXX. Further Investigations of the Formation of Buds and Leaves in *Dryopteris aristata* Druce

BY

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(*Department of Cryptogamic Botany, University of Manchester*)

With Plate XX and fifteen Figures in the Text

ABSTRACT

The influence of the shoot apex upon leaf and bud formation in the fern *Dryopteris aristata* has been investigated by further experiments on puncturing the apical cell. When the apical cell group is damaged, leaf primordia, which may be orientated abnormally, continue to be formed on the meristem, but one or more buds may also arise. The observations reported here indicate that a zone at the periphery of the apical meristem is particularly reactive when the apical cell group is damaged, the majority of buds being induced in this region. The extent of damage to the apex may affect the sequence of organogenesis: when damage is extensive buds tend to be formed immediately, subsequent primordia developing as leaves; when the damage is confined to the apical cell, or extends to only a few of its segments, bud formation tends to be delayed.

It is concluded that the effect of the apical cell on organ formation is exercised through the growth and organization of the apex as a whole.

INTRODUCTION

WHEN an intact shoot apex of *Dryopteris aristata* (*D. austriaca*) is laid bare, the only primordia present are those of leaves. Buds are never present on the intact apical meristem, the lateral shoots which may develop later originating in mature regions of the shoot from detached meristems (Wardlaw, 1943). If, however, the site of a new primordium, i.e. I_1, or of a very young leaf primordium, i.e. P_1 or P_2, is isolated from the upper part of the apical meristem by a wide and deep tangential incision, a bud will usually be formed (Wardlaw, 1949*a*; Cutter, 1954). Buds may also be formed at various points on the meristem if the apical cell is damaged. The buds thus induced are characterized by their early attainment of large size and by their solenostelic vascular system. The large size and radial symmetry of these bud meristems is in striking contrast to the relatively small size and dorsiventral symmetry of young leaf primordia. Questions of considerable interest and importance in morphogenesis relate to the action of the conspicuous shoot apical cell and its recently formed segments (described in these papers as the *apical cell group*) in determining the inception, symmetry, and orientation of leaf primordia, and the inhibition of buds, in the normal development.

An over-simplified hypothesis relating to these phenomena might take the

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]
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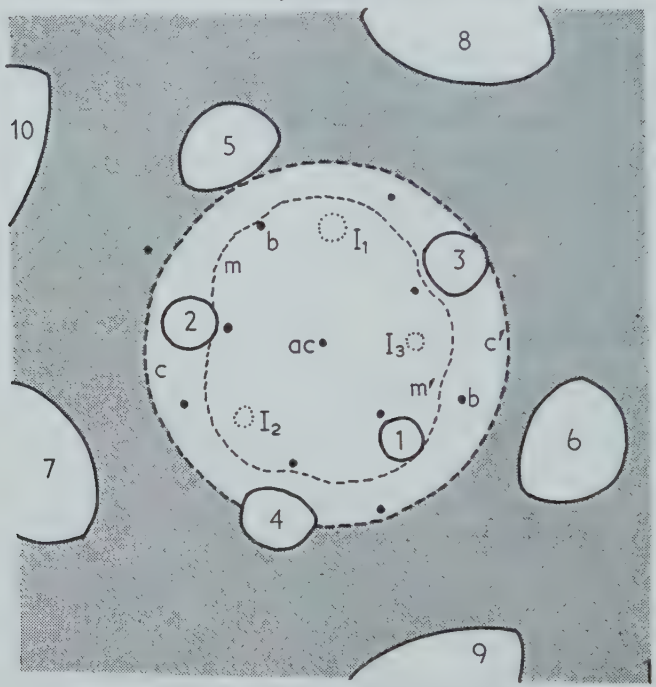
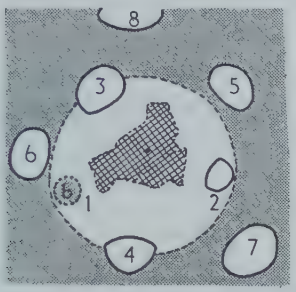
form that the dorsiventrality of leaves is due to the localized inhibitory action, on the adaxial side of primordia, of a downwardly moving growth-regulating substance from the apical cell group; when this is precluded, e.g. by a wide and deep tangential incision, the formation of lateral members of radial symmetry might follow as a natural consequence. That a simple and direct explanation of this kind is not likely to be adequate is shown by the fact that,



TEXT-FIG. 1. Diagrammatic representation of the apex of *Dryopteris aristata* as seen from above, showing the positions of the primordia P_1–P_{10}, the sites of primordia I_1, I_2, I_3 which have not yet appeared, and the sites of the interfoliar bud rudiments. P_3 is likely to have been determined as a leaf at this stage with its two-sided apical cell. If P_2 has not yet been determined as a leaf, it is likely to form a large bud on the destruction or isolation of the apical cell. It may be indicated as the most reactive primordium or site capable of being transformed into a bud. P_1 is the next most reactive as determined by its proximity to the actively growing region of transition to the sub-apical region (×20).

if the apical cell is punctured, both leaves and buds may be formed on the meristem (Wardlaw, 1949*b*, 1950). Moreover, it has been possible to induce bud formation in apices in which the apical cell was intact and not completely separated off from young primordia or primordium sites by deep tangential incisions (Wardlaw, 1955*a*, 1955*b*). In these investigations the conclusion was reached that the orientation and symmetry of a leaf primordium cannot be referred to the direct action of a growth-regulating substance moving basipetally from the apical cell group, but rather that these and other characteristic developments are mediated through the organization and physiological activity of the apex as a whole, the intact apical cell being a central and essential element of the system. Deep and wide tangential incisions, or destruction of

the apical cell, both of which will bring about radical modifications in the normal acropetal and basipetal movements of metabolic substances, may be expected to be attended by notable changes in the distribution of growth in the meristem, one characteristic result being seen in the formation of buds. As



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Text-figs. 2, 3. Apex with comparatively large amount of necrosis round the punctured apical cell (cross-hatched).

Text-fig. 2. Primordium P_1 is becoming a bud, ten days after beginning of experiment.

Text-fig. 3. Thirty days later: a bud has also arisen above the axil of P_5. No primordium has been formed at I_1, but a normal leaf primordium has appeared in the I_2 site. I_3–I_5 are somewhat out of their normal phyllotactic positions.

I_3 and I_5 are directed away from the shoot apex; I_4 appears to be a centric leaf, uniformly surrounded by scales ($\times 14$).

a further contribution to this topic an analytical account is now given of organogenic developments in apices of *Dryopteris aristata* in which the apical cell had been punctured or otherwise damaged. In particular, this investigation has focused attention on the relative reactivity of different regions of the apex.

Materials and Methods

The materials consisted of large apices of *D. aristata*. The apical meristem was laid bare and the pieces of rhizome grown in peat as described in earlier

papers in this series. The terminology for leaf sites and primordia is that now generally used in studies of phyllotaxis, Text-fig. 1. Apical cells were destroyed by being lightly or more severely punctured by fine, sharp needles. Specimens in which the apical cell had been slightly damaged in the course of preparation are also included in these records.

Observations on Organogenic Developments

Punctured apices showed considerable differences in the extent of the eventual necrosis produced. In the majority of specimens a group of cells at the apex became necrosed. By using tungsten needles (Cannon, 1941), however,

Table 1

The Development of Buds and Leaves in Punctured Shoot Apices

Primordium or site.	Number which developed as buds.	Number which developed as leaves.	Total buds.	Total leaves.
P_2	3	22		
P_1	6	20		
I_1	4	25	14	108
I_2	0	23		
I_3	1	18		
Above axil of				
P_7	2	—		
P_6	1	—		
P_5	4	—		
P_4	0	—	9	—
P_3	2	—		
P_2	0	—		
P_1	0	—		
I_1	2	—		
I_2	4	—	8	—
I_3	1	—		
I_4	1	—		

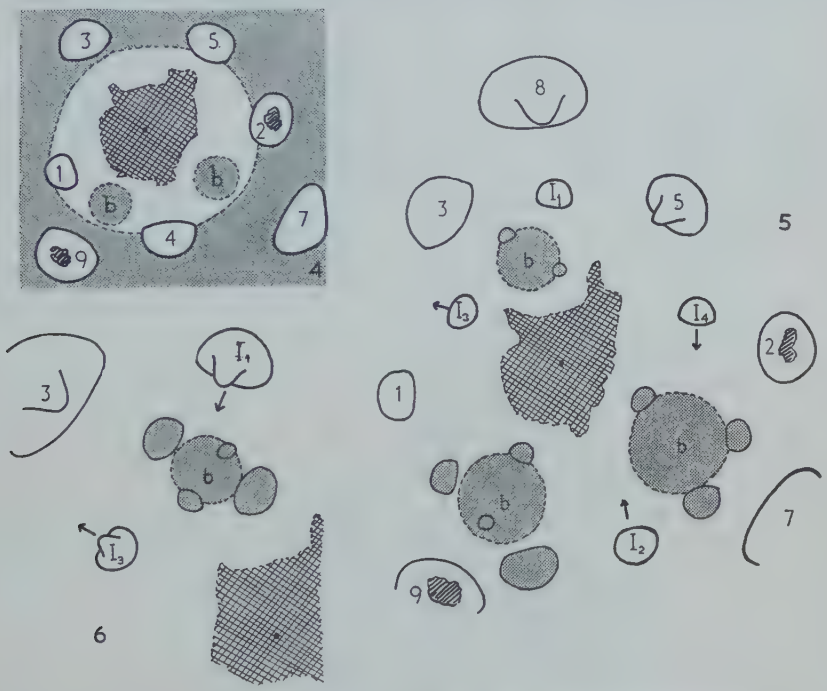
occasional specimens were obtained in which the apical cell was very slightly damaged, so that it alone became necrosed (Pl. XX, Figs. 1–3). Even in such specimens, however, parenchymatization of neighbouring cells eventually took place and scales were formed around the damaged area (Wardlaw, 1949*b*; see also Pl. XX, Fig. 1). Wound tissue was formed beneath the necrotic tissue (Pl. XX, Figs 1–3).

The results of these experiments confirm and extend those of Wardlaw (1949*b*; 1950): leaves continued to be formed in normal phyllotactic sequence after the shoot apex was damaged, and one or more buds arose at various points on the apex, Table 1.

Table 1 gives a summary of the organogenic development, in twenty-six punctured shoot apices, of primordia P_1 and P_2 (which were present at the beginning of the experiment), of I_1–I_3 which developed subsequently, of bud sites above the axils of the older primordia, P–P_7, and of late-formed buds

which eventually appeared above the axils of the new leaf primordia I_1–I_4. The reader will recall that buds are never present in the intact fern shoot apex.

These records show that P_1 and P_2 may be transformed into buds, that leaf-sites I_1–I_3 may give rise to them, that they may be formed above the axils



Text-figs. 4–6. Apex with considerable necrosis round punctured apical cell.

Text-fig. 4. Four days after experimental treatments, buds are being formed above the axils of P_7 and P_9.

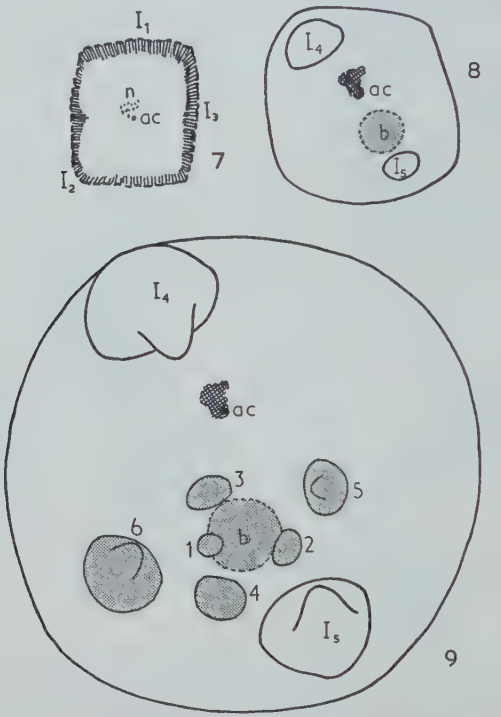
Text-fig. 5. Twenty-three days later: the two original buds have now become conspicuous, a bud has appeared above the axil of P_3, and new leaf primordia I_1–I_4 have appeared. At this stage I_1 and I_2 appear to be normally orientated towards the original shoot apex, but I_4 has become orientated towards the bud of P_7, while I_3 is directed outwards and away from the shoot apex.

Text-fig. 6, as observed 15 days later, shows that I_1 has, in fact, become orientated towards the bud of P_3, while I_3 is clearly directed outwards (× 14).

of the top whorl of primordia, P_3–P_7, and that occasional late-formed buds may be observed high up on the apex, i.e. above the axils of I_1–I_4. The records also show that in punctured apices many more leaves were formed than buds, the ratio being approximately 3·5 : 1. This record, moreover, only deals with new primordia up to I_3; but, in fact, in some apices new leaf primordia including I_5 and I_6 were observed. Table I also shows that a majority of the induced buds arise in or near the region of transition from the base of the apical meristem to the sub-apical region, Text-fig. 1. Here it may also be noted that buds are characteristically formed from I_1, I_2, and I_3 and above the

axils of the top whorl of primordia, i.e. P_1–P_7, when the apices of these primordia as well as the shoot apex have been destroyed (Wardlaw, 1950).

Where the apical cell was destroyed with minimal disturbance, the conical shape of the apex was usually maintained for a considerable time and leaves continued to be formed. In fact, in some of these apices no buds were observed. This bears out earlier observations (Wardlaw, 1949*b*). But where



Text-figs. 7–9. Text-fig. 7. An apex isolated by four vertical incisions after 18 days; some necrosis was apparent in a group of cells close to the apex.

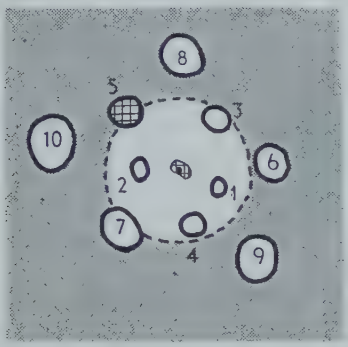
Text-fig. 8. Eight days later, leaf primordia have appeared in what appear to be the I_4 and I_5 positions; a bud is just beginning to form above the axil of I_5; the apex has now become necrosed.

Text-fig. 9. Twenty days later, the necrosed apex has been pushed to one side by the extensive development of the bud and the whole isolated region has become considerably enlarged (× 14).

the shoot tip was more severely damaged, with extensive wound healing and flattening of the cone, bud formation was conspicuous. Even in these specimens, however, some leaf primordia were observed. Some of these primordia, e.g. I_4 or I_5, became orientated in relation to the induced buds in proximity, or actually faced and curved outwards and away from the original shoot tip, Text-figs. 2–6.

Records of special interest are illustrated in Text-figs. 2–15 and Plate XX. In Text-figs. 2–3, primordium P_1 has been transformed into a bud and a second bud has arisen above the axil of P_5. At a later stage it was noted that I_3 and I_5

were directed away from the shoot apex. In Text-figs. 4–6, buds have been formed above the axils of P_7 and P_9. Later a bud appeared above the axil of P_3; leaf primordium I_1 eventually became orientated in relation to this bud,



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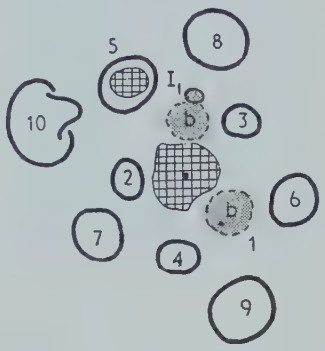

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TEXT-FIGS. 10–12. An apex in which the damage resulting from the puncture was extensive.

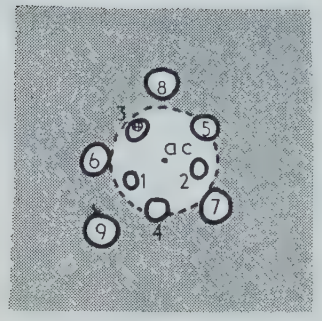
Text-fig. 10. On the day of the operation.

Text-fig. 11. Twenty-one days later. P_1 and I_1 are developing as buds. The damage to the apex is now extensive.

Text-fig. 12. Nineteen days later. The buds at I_1 and P_1 have given rise to leaf primordia, and P_2 has developed as a double leaf (×14).

Text-fig. 6. Similarly I_4 has come into relation with the bud of P_7. I_3 eventually became directed away from the main shoot apex, Text-figs. 5, 6.

In Text-figs. 7–9 an apex was isolated by four deep vertical incisions and after some time leaf primordia began to appear in the I_4 and I_5 positions. By this time some necrosis in cells close to the apical cell had spread to it. A bud appeared above the axil of I_5 and rapidly grew to large size, displacing the original necrosed apical cell into a lateral position, Text-fig. 9.



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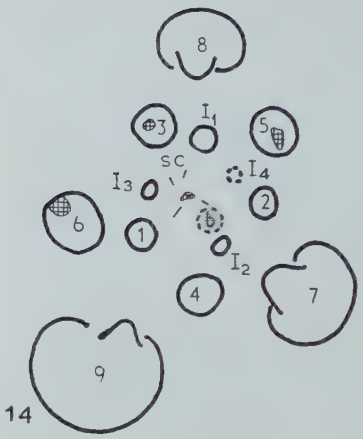

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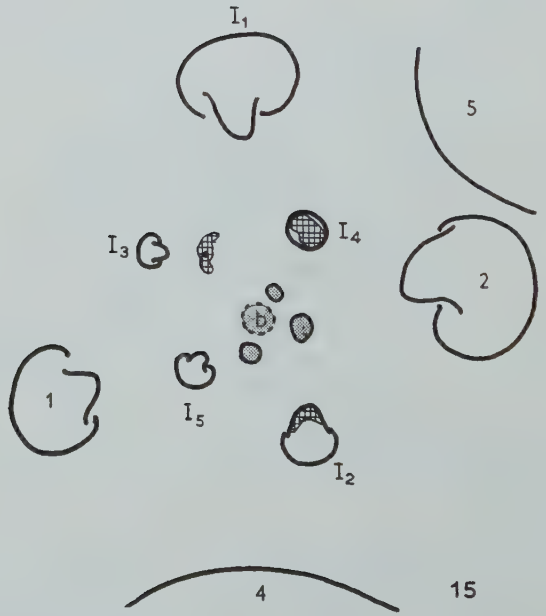

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TEXT-FIGS. 13–15. An apex in which the damage was confined to a single cell.

Text-fig. 13. On the day of the operation.

Text-fig. 14. Twenty-eight days later. I_1–I_4 have developed as leaves, and a bud is arising axillary to I_2. Scales are present around the damaged cell (sc.).

Text-fig. 15. Twenty-six days later. I_5 has also developed as a leaf. The bud axillary to I_2 has given rise to three leaf primordia (× 14).

Text-figs. 10–12 illustrate a case in which the damage resulting from the puncture was extensive. In this specimen P_1 and I_1 developed as buds, and P_2 as a double leaf (Text-fig. 12). (Double leaves may arise on apices which have not been subjected to experimental treatment.) By contrast, in a specimen in which only the apical cell was damaged, P_1 and P_2 continued to develop as leaves; I_1–I_6 also developed as leaves, and a single bud arose high on the apical cone in a position axillary to I_2 (Text-figs. 13–15). Plate XX, Fig. 1, illustrates, in longitudinal section, another apex in which only a single cell was damaged by the puncture; in this case I_1–I_3 developed as leaves, and a bud was formed axillary to I_2, high up on the apical cone. These observations suggest that the amount of damage to the apical cone may be important in subsequent organogenesis.

Discussion

The records of the further development in apices of *D. aristata* in which the shoot tip has been more or less severely damaged, with subsequent necrosis, wound healing, parenchyma and scale formation, show that both leaf and bud formation may continue simultaneously for some time. In general, leaf formation is characteristic of the less active upper region of the apical cone, whereas bud formation is typical of the region of more active growth towards the base of the cone. Any simple hypothesis along the lines that, in the normal intact apex, bud formation is inhibited by the direct action of a growth-regulating substance proceeding from the apical cell group, or that leaf dorsiventrality results from the localized action of this substance on the adaxial sides of young primordia, seems unlikely to prove satisfying. Also, a hypothesis of the kind that the intact apical cell group produces a substance which promotes leaf formation but inhibits bud formation, does not seem to be in accord with the experimental observations. What this investigation has done is to focus attention on the importance of the organization of the shoot apex, including its histology, characteristic shape, and distribution of growth, in determining organogenesis. The continuing formation of the shoot axis and the formation of dorsiventral lateral members are the normal result of the activity of the intact apex. But when the apical cell group is damaged or isolated, very marked growth activity ensues in the basal region of the cone and buds may be formed. In the upper region of such a damaged apex, however, the normal slow rate of growth and the conical character of the apex may be maintained and leaf primordia continue to be formed. The eventual orientation of these primordia, however, may be outwards or towards a neighbouring bud.

In a closer analysis of the organogenic developments recorded in this paper, the following observations seem to be pertinent:

(1) There is a steady acceleration in the rate of growth from the apex to the base of the normal intact apical cone, the zone of transition to the sub-apical region being marked by a rapid acceleration in the transverse growth rate (Wardlaw, 1955*a*, 1955*b*). Histologically this transition region is one in which there is extensive development of parenchyma in the pith and cortex of the

shoot and leaf bases, especially on the abaxial side. There is also an abundant outgrowth of scales. These several developments, which may be regarded as an indication of the nutrition available at this level in the shoot, and of the capacity of cells to use such nutrition, are in marked contrast to the embryonic or meristematic character of the cells of the conical region of the apex above, and, presumably, their essentially proteinaceous metabolism.

(2) Leaf primordia, which first become visible a little above the base of the apical cone, also show a steady acceleration of growth and become progressively more conspicuous as they occupy positions farther down the cone and on the sub-apical region. P_3 is thus growing more rapidly than P_2, P_2 than P_1, and P_1 than the primordium site I_1. In that P_3 is usually already determined as a leaf, with its characteristic large two-sided apical cell, and P_2 frequently so at the beginning of an experiment, these primordia are of less interest from the point of view of bud induction than P_1. The important point that has emerged from the present investigation is that the region on the meristem about the level of P_1–P_2 is one of great reactivity when the apical cell group is destroyed or isolated; a majority of the induced buds, and those first to appear, arise about this level. (The buds which have been observed near the summit of the apex, e.g. above the axil of I_3, are typically late in formation.)

In that a bud primordium (*a*) shows as much growth on the adaxial as on the abaxial side, in marked contrast to a leaf primordium, and (*b*) soon forms a large and conspicuous mound-like apical meristem, very considerably larger than that of a leaf primordium at the P_1 or P_2 stage of development, it may be inferred that destruction or isolation of the apical cell group is attended by radical changes in the distribution and utilization of the substances involved in the development of primordia in the reactive basal region of the cone. The regulated development of the normal intact fern apex, including the maintenance of its conical shape and morphogenetic activity, is undoubtedly a very complex phenomenon: it may be regarded as the resultant of the many processes which are involved in the growth and initial differentiation of embryonic cells. These processes may include: (i) the basipetal movement of growth-regulating substances from the apical cell group; (ii) competition among the constituent cells of the apical meristem, including those of primordia and primordium sites or growth centres, for the materials required in protein synthesis and other special metabolic activities of embryonic cells; and (iii) the differential utilization by cells at different levels *en route* of the nutrients being drawn up to the apex from below, and the progressively diminishing supplies of such nutrients (Wardlaw, 1955*a*, 1955*b*). This complex system will be radically modified if the apical cell group is destroyed, e.g. its competitive activity and production of growth-regulating substances will be eliminated. Again, the more or less extensive formation of parenchyma and scales high up on a damaged apical meristem, or round any apical incision—developments never observed in the intact apex—are indicative of a utilization of metabolic materials closely comparable with that on the abaxial side of leaves at the base of the cone. In short, when the apical cell group is destroyed, or isolated by

a deep tangential incision, the several changes apparently include a levelling up of the rates of growth on the adaxial and abaxial sides of primordia situated near the base of the cone and their more rapid growth. Thus, if primordium P_1 or P_2 has not already been determined as a leaf, it is likely to develop as a bud, with radial symmetry, a large meristem, and a conspicuous solenostelic vascular system. The sites of interfoliar bud primordia at this level, which in the normal intact apex consist of small areas of meristematic cells in an inhibited condition, and of I_1 and I_2, may also become conspicuously active when the apical cell group is damaged, especially if the effects of the adjacent leaf primordia are also eliminated (Wardlaw, 1950). On the other hand, if the injury in the apical cell region is slight, the formation of wound parenchyma and scales takes place slowly, and the less reactive, and evidently less modified, upper regions of the apical cone may continue to give rise to leaf primordia in normal phyllotactic positions. Some of these show the normal orientation towards the shoot apex; but others apparently come under the organizing influence of the adjacent bud meristem and become orientated towards it. Lastly, some primordia develop as outwardly or downwardly orientated leaves, these affording evidence of an increased rate of growth on the adaxial side which may well be related to the extensive formation of parenchyma and scales at the injured shoot tip.

This further analysis of the formation and distribution of leaf and bud primordia in ferns lends support to the view already advanced by Wardlaw (1955*a*, 1955*b*), that while the apical cell is the central and essential component of the normal, organized, intact apical meristem, its action in determining the position, orientation, and symmetry of leaf primordia is not a direct one, but rather is mediated through the organization of the meristem as a whole.

The writers have pleasure in thanking Mr. E. Ashby and Mr. G. Barker for the photographic illustrations. This work was carried out during the junior author's tenure consecutively of a Berry Scholarship, awarded by the University of St. Andrews, and a Carnegie Scholarship, for which grateful acknowledgement is made.

Literature Cited

Cannon, H. G., 1941: A note on Fine Needles for Dissection. J. Roy. Micr. Soc., ser. 3, lxi. 58.

Cutter, E. G., 1954: Experimental Induction of Buds from Fern Leaf Primordia. Nature, London, clxxiii. 440.

Wardlaw, C. W., 1943: Experimental and Analytical Studies of Pteridophytes. II. Experimental Observations on the Development of Buds in *Onoclea sensibilis* and in Species of *Dryopteris*. Ann. Bot., n.s., vii. 357.

—— 1949*a*: Experiments on Organogenesis in Ferns. Growth (supplement), xiii. 93.

—— 1949*b*: Further Experimental Investigations of the Shoot Apex of *Dryopteris aristata* Druce. Phil. Trans. Roy. Soc. B, ccxxxiii. 415.

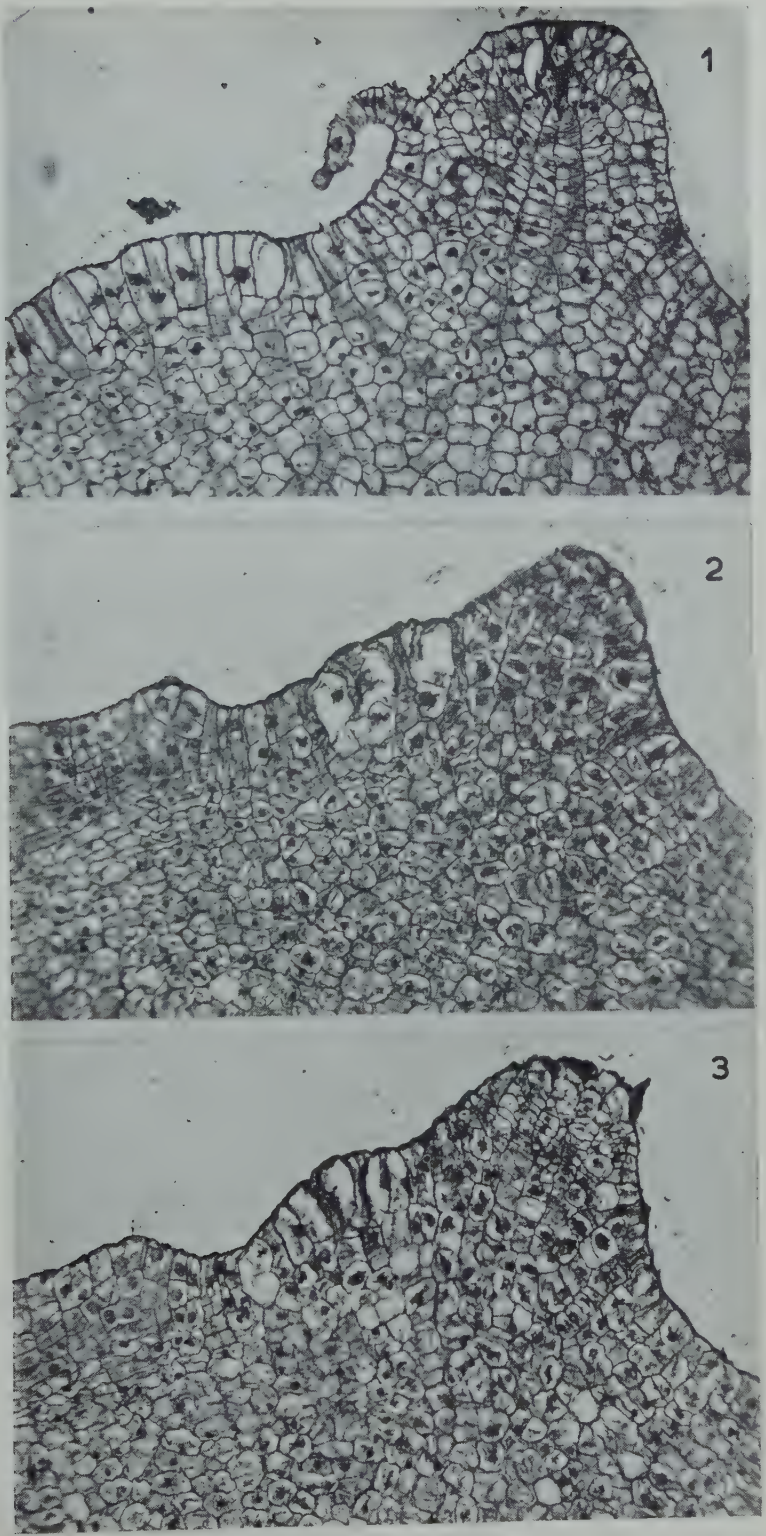
—— 1950: Experimental and Analytical Studies of Pteridophytes. xvi. The Induction of Leaves and Buds in *Dryopteris aristata* Druce. Ann. Bot., n.s., xiv. 435.

WARDLAW, C. W., 1955*a*: Experimental Investigation of Leaf Formation, Symmetry and Orientation in Ferns. Nature, London, clxxv. 115.
—— 1955*b*: Experimental and Analytical Studies of Pteridophytes. XXVIII. Leaf Symmetry and Orientation in Ferns. Ann. Bot., N.S. xix. 389.

EXPLANATION OF PLATE XX

FIG. 1. Longitudinal median section of an apex in which the apical cell alone was damaged. Files of cells (wound tissue) can be seen below the necrosed apical cell and a scale, curving downwards, can be seen on the left. The bud meristem, on the left, lies above the axil of I_2 (×100).

FIGS. 2 and 3. Longitudinal median sections of an apex damaged as in Fig. 1. Files of wound tissue can be seen below the necrosed distal region and a conspicuous, late-formed bud has appeared above the axil of I_3 (×112).



C. W. WARDLAW *and* E. G. CUTTER

not controlled by either the amount of light or mean temperature, (ii) the relative growth rate of the root and the root-weight ratio are positively linked only with temperature, (iii) the rate of leaf growth either in area or weight together with the net assimilation rate (area basis) are positively dependent on light alone, (iv) the net assimilation rate (weight basis) and the relative growth rates of the whole plant and the stem are directly and positively correlated with both temperature and light, and (v) the leaf-area ratio, the ratio of leaf area to leaf weight and the stem-weight ratio are depressed by increasing light but augmented by rising temperature. In the individual regressions for net assimilation rate (area and weight), the relative growth rates of the whole plant, stem and leaf weight, and the ratios of stem weight and leaf area to leaf weight the percentage variation accounted for ranged from 47 to as high as 91 per cent.

The implication of these findings in relation to experiments in controlled environmental chambers are discussed.

INTRODUCTION

ALTHOUGH the concepts of growth analysis, first formulated more than thirty-five years ago, provided a new and critical approach to ecological and physiological studies of environmental factors surprisingly little information is as yet available on the relationships between growth and development and seasonal changes in light intensity and temperature. In fact, for only one plant—the tomato—grown under greenhouse conditions, have continuous records been made of the variations in net assimilation rate and relative growth rate of plants with a comparable stage of vegetative development at the beginning of each set of observations (Goodall, 1945). It is true that a number of other workers, e.g. Gregory (1926), Williams (1939), and Watson (1947), have linked variations in net assimilation rate with fluctuations in environmental factors, but where observations have also been made on the relative growth rate the results refer to the changes which take place during different periods of the life-cycle.

For barley, Gregory (1926) found that variations in the net assimilation rate up to the time of maximal leaf area were governed by changes in the environment and not by internal physiological factors, while Crowther (1934) was of the opinion that until the initiation of flowering in cotton the assimilation rate is independent of the stage of development. On the other hand, Williams (1936, 1937), working with oats, and Ballard and Petrie (1936) with sudan grass, concluded that there was a steady decrease in the net assimilation rate throughout the vegetative phase. However, Watson and Baptiste (1938)

record that over periods of ten weeks the net assimilation rates of mangolds sown on different dates during the season exhibited little ontogenetic drift. More recently, Williams (1946) in a further examination of the concept of net assimilation rate concluded that in general the net assimilation rate is least variable in the early vegetative phase irrespective of whether it is measured in terms of leaf area, leaf weight, or, if the nitrogen level is in short supply, of leaf protein. In this connexion Blackman and Wilson (1951*a*) subjected *Helianthus annuus* in different stages of early vegetative development to a range of light intensities between 0·25–1·0 daylight and found that the net assimilation rates were not dependent on the age of the plants though there were significant differences in the relative growth rates since the leaf-area ratios were affected both by light intensity and the stage of development.

From these findings it is therefore apparent that in a critical study of the effects of seasonal changes in light intensity and temperature on net assimilation rate and relative growth rate, plants of a similar physiological status are demanded at the beginning of each experimental period. It also follows that the errors due to ontogenetic drifts will be minimal when young plants with no senescent leaves are employed.

In his investigation Goodall (1945) estimated the diurnal changes in light intensity by taking readings at half-hourly intervals by means of a holophane lumeter. Watson (1947) obtained a more satisfactory estimate of the total energy received during the day since the Callender recorder employed integrated the rapid fluctuations in intensity which often occur over short periods of time. It should, however, be emphasized that such an instrument integrates not only the visible bands of the spectrum but also the infra-red bands, which are not concerned with photosynthesis. Thus, with data from this type of record in seeking to assess the relative importance of the temperature and light factors it is difficult to disentangle the direct effects of light on growth processes from the indirect heating effects of the thermal bands. Therefore in order to overcome this difficulty a special light-integrating apparatus was constructed which only included in the estimate the range of wave-lengths (4,000–7,000 Å) important for photosynthesis.

At the same time, so as to minimize the biological errors, young vegetative sunflower plants of the same initial size and stage of development were selected for each of the consecutive experimental periods. To make the investigation as complete as possible, besides determining the relative growth rate and net assimilation rate of the whole plant, the plants were subdivided into root, stem, and leaves so that any influence of the environmental factors on the proportionate growth of the several parts could also be studied.

Experimental Methods

Pot-culture techniques. The methods of sampling and the pot culture techniques have already been described in previous papers (Blackman and Wilson, 1951*a*, 1954). Briefly, seed of *H. annuus* (variety Pole Star) was sown in the open in a 1:1 mixture of soil and sand contained in pots of 10-inch

diameter. To ensure that the levels of nutrient supply and water were not limiting growth a fertilizer mixture containing nitrogen, phosphorus, and potassium was added to the pots while they were watered whenever the occasion demanded. Excess seed was sown in each pot and the seedlings thinned twice to ensure uniformity; in addition, at the beginning of each experiment individual pots were further matched by eye into a series of groups, each of which constituted a block.

In both 1950 and 1951, a week was selected as the standard duration of the individual experiments and in the consecutive experiments covering the period May to September the aim was to start each experiment with plants of a uniform morphological status. The criterion selected was that the third pair of true leaves should be visible: to achieve this batches of pots were sown throughout the season at intervals of a few days and the batch nearest to the standard was chosen at the beginning of each week. The replication was fivefold and six plants were left in each pot.

For the initial and final samplings at the beginning and end of the week a pot was treated as a single unit. The plants were divided at ground level to constitute the root and the shoot. The root system was recovered by washing the soil-sand mixture through a fine sieve, while the leaves were separated from the shoot at the base of each lamina so that the stem also included the petioles. A sub-sample of leaves was then taken for the measurement of leaf area, which was accomplished by 'blue printing' the leaves and subsequently measuring the areas with a planimeter. All root and shoot samples were dried at 100° C. for 24 hours and weighed.

Light and temperature records. The design, construction, and performance of the integrating light recorder have been described in a previous paper of this series (Blackman, Black, and Martin, 1953). The diurnal amount of light received per day was read off daily.

Temperatures were recorded by means of an adjacent thermograph placed in a Meteorological Office Stevenson screen set up at the same level as the plants. The thermograph was calibrated over a range of temperatures against a mercury thermometer at the beginning and end of both the 1950 and 1951 seasons. Three types of temperature measurement were abstracted from the record, namely, the mean daily maximum, the mean daily minimum, and the mean diurnal temperature derived by measuring the enclosed area with a planimeter.

Statistical treatment. Since for each sampling occasion estimates of the weights of the whole plant W, the root R, the stem S, the leaves L, and the total leaf area A were obtained, it is evident that much time could be spent in studying a great many derived variables. Although the analysis of a large number of variables should always be treated with caution it was thought that most value would accrue from selecting the following twelve variables, namely, the ratios

$$\frac{R}{W}, \frac{S}{W}, \frac{L}{W}, \frac{A}{W}, \frac{A}{L},$$

the net assimilation rates on a leaf-weight and leaf-area basis

$$\frac{1}{L}\cdot\frac{dW}{dt},\quad \frac{1}{A}\cdot\frac{dW}{dt},$$

and the relative growth rates

$$\frac{1}{W}\cdot\frac{dW}{dt},\quad \frac{1}{R}\cdot\frac{dR}{dt},\quad \frac{1}{S}\cdot\frac{dS}{dt},\quad \frac{1}{L}\cdot\frac{dL}{dt},\quad \frac{1}{A}\cdot\frac{dA}{dt}.$$

Functional relationships within these variables are as follows:

$$1 = \frac{R}{W}+\frac{S}{W}+\frac{L}{W},$$

$$\frac{A}{L} = \frac{A}{W}\div\frac{L}{W},$$

$$\begin{aligned}\frac{1}{W}\cdot\frac{dW}{dt} &= \frac{R}{W}\times\frac{1}{R}\cdot\frac{dR}{dt}+\frac{S}{W}\times\frac{1}{S}\cdot\frac{dS}{dt}+\frac{L}{W}\times\frac{1}{L}\cdot\frac{dL}{dt}\\ &= \frac{L}{W}\times\frac{1}{L}\cdot\frac{dW}{dt}\\ &= \frac{A}{W}\times\frac{1}{A}\cdot\frac{dW}{dt}.\end{aligned}$$

Before the statistical analyses were carried out various methods of calculating the dependent variables were studied. There was, by and large, little difference between the values obtained by the several methods that have been suggested in the past (see Williams, 1946). In general there is little point in applying very refined methods of calculation when the accuracy of the basic data is inevitably limited.

The method originally advocated by Fisher (1921) was employed for the relative growth rate of the whole plant:

$$\frac{\log_e W_1-\log_e W_0}{t_1-t_0}$$

where W_0 and W_1 represent the values at the initial and final sampling occasions (t_0 and t_1) of the weight of the whole plant. The relative growth rates of the parts of the plant were found similarly.

For the leaf-area assimilation rate the following formula[1] was used:

$$\left(\frac{\log_e W_1-\log_e W_0}{t_1-t_0}\right)\div\frac{1}{2}\left(\frac{A_0}{W_0}+\frac{A_1}{W_1}\right)$$

[1] It is of interest to note that in practice this formula and the more usual

$$\left(\frac{W_1-W_0}{A_1-A_0}\right)\times\frac{(\log_e A_1-\log_e A_0)}{t_1-t_0}$$

are in close agreement, since

$$\frac{1}{2}\left(\frac{A_0}{W_0}+\frac{A_1}{W_1}\right)\quad\text{and}\quad\left(\frac{A_1}{W_1}-\frac{A_0}{W_0}\right)\times\left(\frac{\log_e W_1-\log_e W_0}{\log_e A_1-\log_e A_0}\right)$$

give almost exactly the same values for leaf-area ratio.

where A_0 and A_1 represent the total leaf area. The leaf-weight assimilation rate was found similarly.

The ratio A/W was calculated by the following formula:

$$\frac{1}{2}\left(\frac{A_0}{W_0}+\frac{A_1}{W_1}\right).$$

The ratio R/W, S/W and L/W were calculated similarly.

The formula for A/L was:

$$\frac{\left(\frac{A_0}{W_0}+\frac{A_1}{W_1}\right)}{\left(\frac{L_0}{W_0}+\frac{L_1}{W_1}\right)}$$

where L_0 and L_1 represent the leaf weight.

The independent variables considered were the mean temperature during the weekly experimental period, the mean daily maximum temperature minus the mean nightly minimum, the total amount of light received, and the 'week number', which was obtained by numbering the experiments negatively backwards and positively forwards from the experiment at midsummer. This term, therefore, reflects the time of year when the individual experiments were undertaken.

For each of the twelve dependent variables separate multiple regression analyses on the independent variables were carried out. The appropriate regression equations were obtained and the percentage of variation accounted for was calculated in each instance. Standard errors were found for each of the equations; these are merely the square roots of the residual mean squares—they are not the standard errors appropriate to predicted values.

The data for the two seasons were initially analysed separately and the possibility of pooling the two years' data was then examined. A dummy variable to represent years was inserted in the analyses of variance and save for the stem-weight ratio it was found that the two seasons' data could be bulked.

Another aspect that demanded investigation was whether some function of total light energy other than a linear one gave a better fit in any of the final multiple regressions. The following technique was employed to investigate this point:

Let x_1 = the total light energy received per week. Then a new variable x_2 was taken where

$$x_2 = 1000\frac{(x_1-\bar{x}_1)}{\bar{x}_1}$$

$$= 1000\,(C\,x_1-1) \text{ say.}$$

This was found to give a set of values lying between $+1$ and -1 for $\frac{x_2}{1000}$.

Now $$x_1 = \left(1+\frac{x_2}{1000}\right) \div C.$$

Hence $$\log_e (x_1) = \log_e \left(1+\frac{x_2}{1000}\right) - \log_e C$$

$$\doteqdot \frac{x_2}{1000} - \frac{1}{2}\left(\frac{x_2}{1000}\right)^2 + \frac{1}{3}\left(\frac{x_2}{1000}\right)^3 - \log_e C.$$

Other functions of x_1 may be treated similarly.
For instance:

$$(x_1)^{\frac{1}{2}} = \left(1+\frac{x_2}{1000}\right)^{\frac{1}{2}} \times C^{-\frac{1}{2}}$$

$$\doteqdot \left[1+\frac{1}{2}\left(\frac{x_2}{1000}\right) - \frac{1}{8}\left(\frac{x_2}{1000}\right)^2 + \frac{1}{16}\left(\frac{x_2}{1000}\right)^3\right] \times C^{-\frac{1}{2}}.$$

Hence the nature of the light-intensity relationship may be determined by calculating the regressions of the dependent variables on x_2, x_2^2, &c. These regressions were obtained and in no case was it found necessary to use any powers of x_2 other than x_2 itself. From these results it was concluded that neither a logarithmic nor a square root transformation of the light data gave any better fit than the original untransformed records.

For temperature there was no indication of a departure from a general linear trend for any of the variables.

Experimental Results

Net assimilation rate. The data for the seasonal variation in the net assimilation rate, both on a leaf-area and a leaf-weight basis, together with the quantity of light received and the mean temperature during the experimental period, are set out for the 1950 and 1951 series in Figs. 1 and 2. It is evident that in both years there is a similar seasonal trend, the net assimilation rates are low both in May and from the middle of August onwards, while the highest figures are recorded in June and July. Inspection also shows that there is a clear correlation between the fluctuations in the assimilation rate and the light energy received. On the other hand, the variation in the rate based on leaf area differs somewhat from that based on leaf weight and this is most noticeable when the rate starts to fall in August, i.e. under conditions when the decline in the diurnal amount of light is not matched by a corresponding fall in temperature.

With such divergences it is not therefore unexpected that over the whole series the regression linking net assimilation rate with the light and temperature factors is dependent on the criterion of expression.

Net assimilation rate (g./dm.^{2}/day)

$$= 0{\cdot}0014 + 0{\cdot}4243\, L \times 10^{-6}$$

S.E. $= \pm 0{\cdot}0136$, percentage variation accounted for $= 67$.

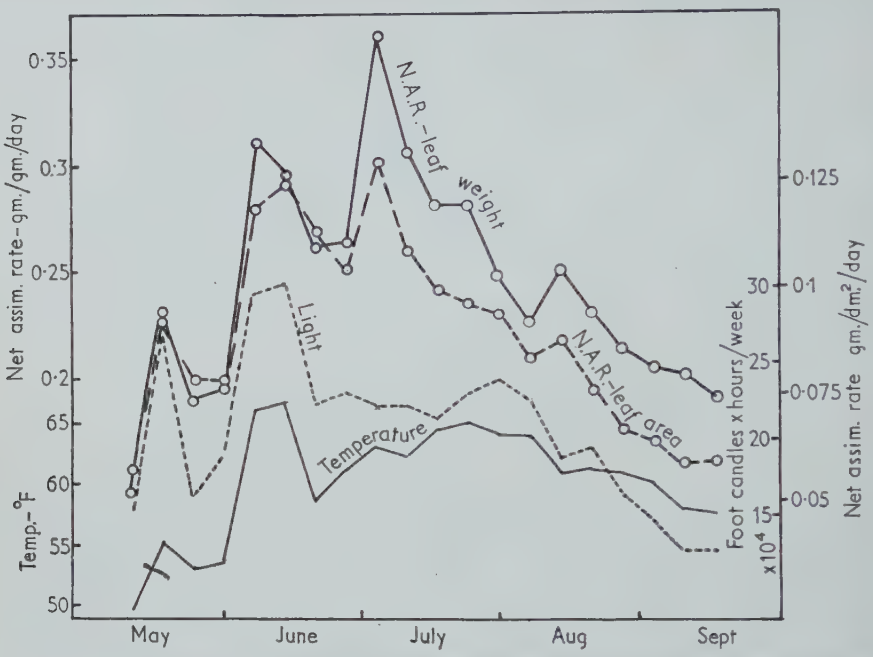


FIG. 1 (1950). Seasonal changes over successive weekly periods in diurnal mean temperature, daylight, and net assimilation rate. Rate expressed on basis of both leaf area and leaf weight.

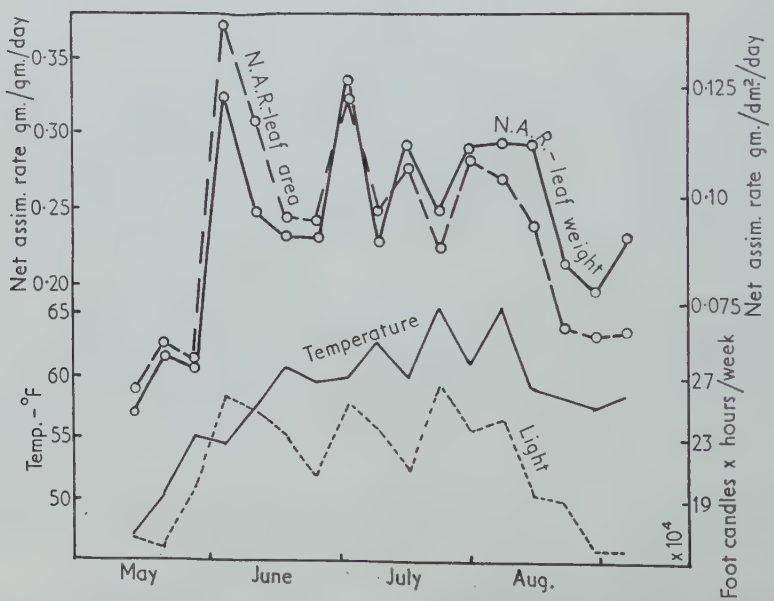


FIG. 2 (1951). Seasonal changes over successive weekly periods in diurnal mean temperature, daylight, and net assimilation rate. Rate expressed on basis of both leaf area and leaf weight.

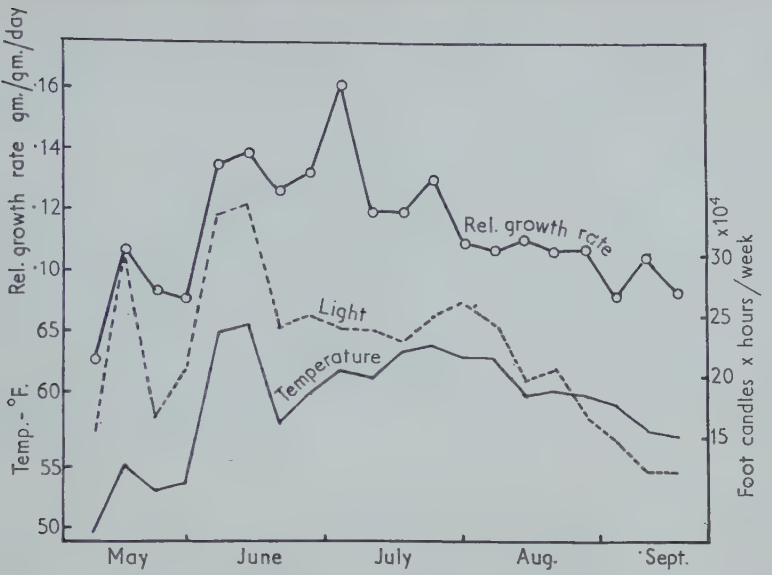


FIG. 3 (1950). Seasonal changes over successive weekly periods in diurnal mean temperature, daylight, and relative growth rate of the whole plant.

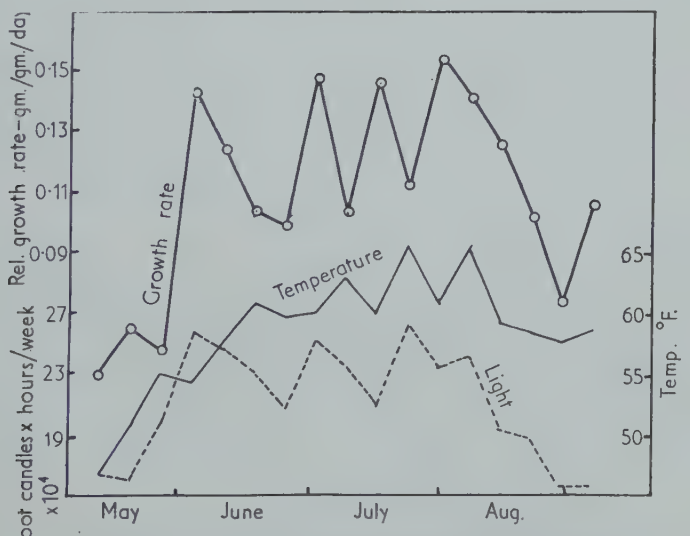


FIG. 4 (1951). Seasonal changes over successive weekly periods in diurnal mean temperature, daylight, and relative growth rate of the whole plant.

From equation (1)[1] it is apparent that on the basis of leaf area the fluctuations show a close linkage with changes in light radiation.

Net assimilation rate (g./g./day)

$$= -0{\cdot}2003 + 0{\cdot}005659T + 0{\cdot}5145L \times 10^{-6} \qquad (2)$$

S.E. $= \pm 0{\cdot}0375$, percentage variation accounted for $= 59$.

[1] L = total light energy received per week, expressed as foot candles × hours.

On the other hand, equation (2)[1] demonstrates that on a leaf-weight basis assimilation is dependent both on light and the mean temperature. It is to be observed that in these and the subsequent equations no term for the diurnal fluctuation in temperature has been included, since the statistical analyses showed that the effects were of little account.

Relative growth rate. The data for the relative growth rates of the whole plant, together with the corresponding temperature and light records, are set out in Figs. 3 and 4. As would be anticipated, the growth rates in both years are lowest at the beginning and end of the season and maximal in mid-season. For equivalent values of light energy received it is noteworthy that the relative growth rates are higher in August and September than they are in May, and this difference would indicate that in late spring it is temperature rather than light which is limiting growth. That both factors are involved in controlling the seasonal changes in the relative growth rate is evident from equation (3)

$$\text{Relative growth rate (g./g./day)} = -0{\cdot}0838+0{\cdot}002468T+0{\cdot}2299L\times 10^{-6} \qquad (3)$$

S.E. $= \pm 0{\cdot}0192$, percentage variation accounted for $= 51$.

Leaf growth. In describing the statistical procedures it has been pointed out that the net assimilation rate can be looked on as the ratio of the relative growth rate to the mean leaf-area ratio. In consequence, the seasonal changes in the leaf-area ratio will be dependent on the seasonal trends for both the growth rate and the assimilation rate. From Figs. 5 and 6 it is apparent that between May and September there is a steady rise in the leaf-area ratio: in other words, the combination in the autumn of a relatively high temperature with a low light value induces the largest ratio.

When the results for the two years were first analysed separately it was found that the fit of the regression could be improved by including the term week number, see p. 532. On consideration it seemed that the most likely explanation for this improvement was that the reactions to light and temperature during the experimental period were in part controlled by physiological changes induced in the plants before the experiment was started. It will be recalled that to eliminate errors between experiments the standard basis selected was that the plants should have reached a stage when the third pair of leaves were clearly visible. This criterion did not allow for the possibility that the size of the first and second pairs of leaves might be influenced differentially by changes in the environment between emergence and the start of the experiment. Such influences might well affect the initial leaf-area ratio and when in the analysis this ratio was taken into account week number could be discounted. Consequently, the equation covering both years was modified to predict the value of the leaf-area ratio at the end of the week (LAR_F), given the initial ratio (LAR_I) and the light and temperature data.

[1] T = mean temperature during experimental period (°F.).

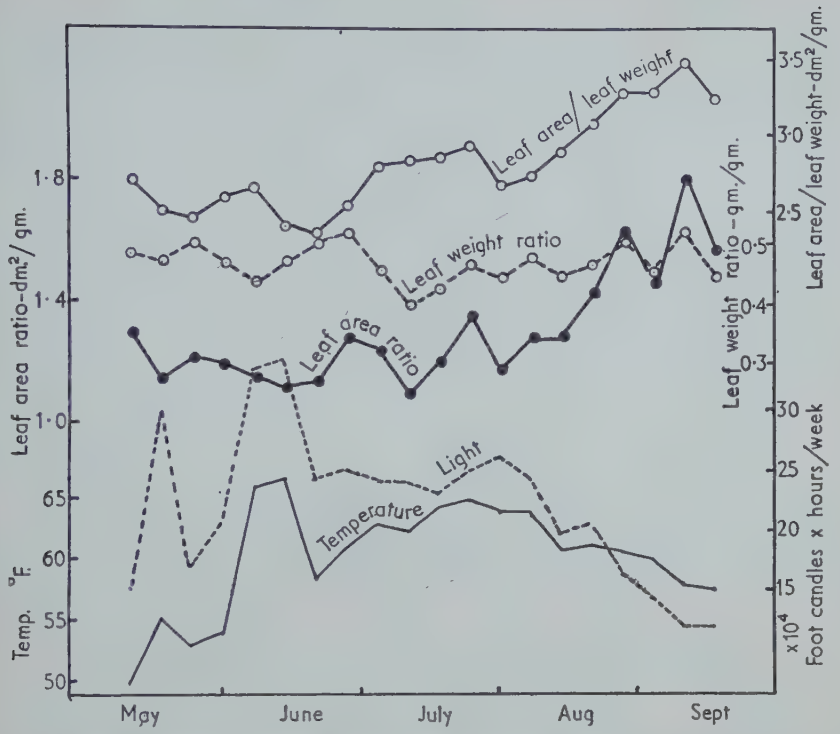


FIG. 5 (1950). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, leaf-area ratio, leaf-weight ratio, and ratio of leaf area to leaf weight.

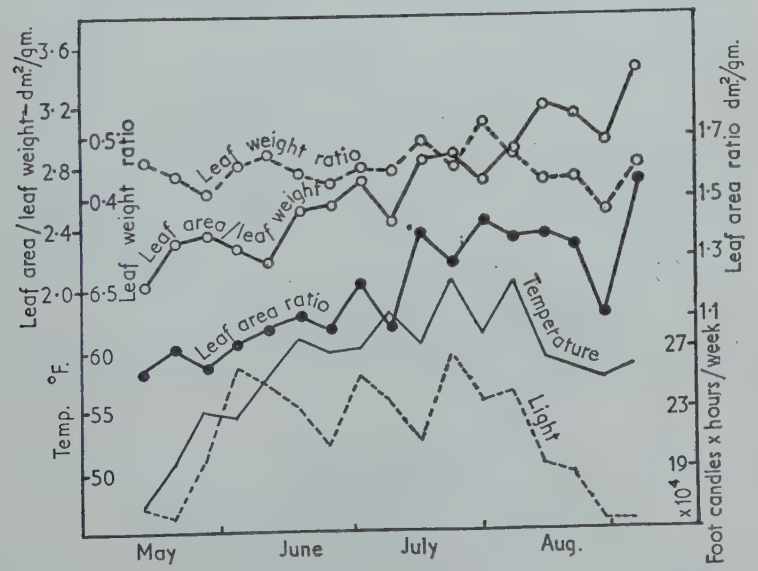


FIG. 6 (1951). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, leaf-area ratio, leaf-weight ratio, and ratio of leaf area to leaf weight.

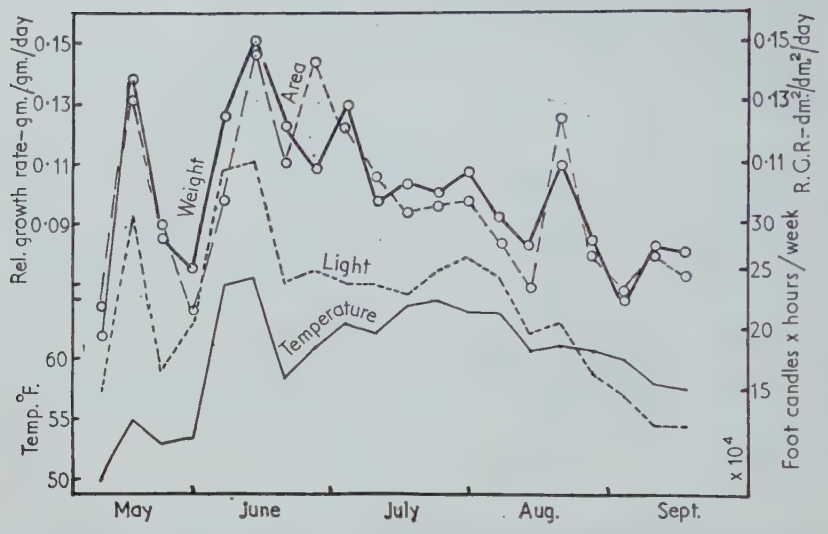


FIG. 7 (1950). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, and the relative growth rates of the leaves on either the basis of area or weight.

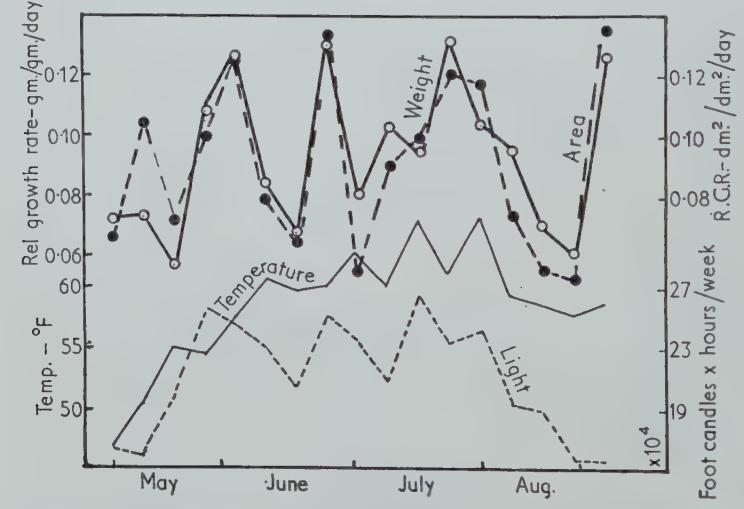


FIG. 8 (1951). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, and the relative growth rates of the leaves on either the basis of area or weight.

Final leaf-area ratio (dm.²/g.)

$$= \mathrm{LAR}_F = 0{\cdot}758 + 0{\cdot}00641T - 0{\cdot}1638L \times 10^{-5} + 0{\cdot}2789\ \mathrm{LAR}_I \qquad (4)$$

S.E. $= \pm 0{\cdot}151$, percentage variation accounted for $= 39$.

The equation indicates that the leaf-area ratio is positively correlated with temperature and negatively correlated with light.

The comparable data for the leaf-weight ratio are also included in Figs. 5

and 6 and it is clear that this ratio is little affected by light and temperature. This conclusion was confirmed in the statistical analyses.

From p. 531 it follows that the differential responses of the leaf-area and leaf-weight ratios must be related to the changes induced by the two environmental factors in the ratio of leaf area to leaf weight. That changes in this ratio are closely linked with changes in the leaf-area ratio is seen in Figs. 5 and 6. The regression is of the same form as equation (4), and a large proportion of the variance of the final ratio can be attributed to the initial value combined with the positive effects of temperature and the negative influence of light.

Final ratio of leaf area to leaf weight (dm.^{2}/g.)
$= (\mathrm{LA/LW})_F = 0{\cdot}679 + 0{\cdot}02907T - 0{\cdot}4290L \times 10^{-5} + 0{\cdot}4325(\mathrm{LA/LW})_I$ (5)
S.E. $= \pm 0{\cdot}206$, percentage variation accounted for $= 71$.

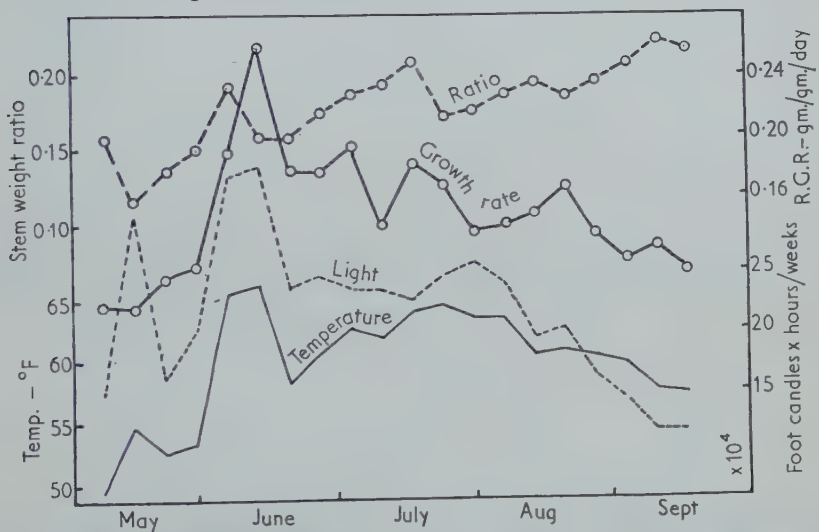


FIG. 9 (1950). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, stem-weight ratio, and the relative growth rate of the stem.

There are two other aspects of leaf growth which can be estimated from the data, namely, the relative growth rates of the leaves both in terms of area and weight. The seasonal trends in both years are also shown in Figs. 7 and 8; there is a general suggestion that the rates are primarily controlled by the light factor. This conclusion is borne out by regressions (6) and (7).

Relative growth rate of leaves (g./g./day)
$= 0{\cdot}0168 + 0{\cdot}3819L \times 10^{-6}$ (6)
S.E. $= \pm 0{\cdot}0183$, percentage variation accounted for $= 47$.
Relative growth rate of leaves (dm.^{2}/dm.^{2}/day)
$= 0{\cdot}0266 + 0{\cdot}3217L \times 10^{-6}$ (7)
S.E. $= \pm 0{\cdot}0232$, percentage variation accounted for $= 28$.

Stem growth. The data for the seasonal variation in the development of the stem can be interpreted in terms of the two selected variables, namely,

the relative growth rate and the stem-weight ratio. The growth rate clearly leaves out of account the extent to which the gain in weight of the stem is dependent on the growth of the whole plant, but on the other hand the ratio is indicative of the partition of substrates between the stem and the remainder of the plant. The seasonal changes in the growth rate and the ratio for both the 1950 and 1951 series are shown in Figs. 9 and 10. For the growth rate the trends, not unexpectedly, are somewhat similar to those for the growth rate of the whole plant (Figs. 3 and 4), in that the rate is maximal under a combination of a high light radiation and a high temperature. The changes in the ratio are quite different; in this instance the ratio is maximal in the autumn when low light is coupled with a relatively high temperature.

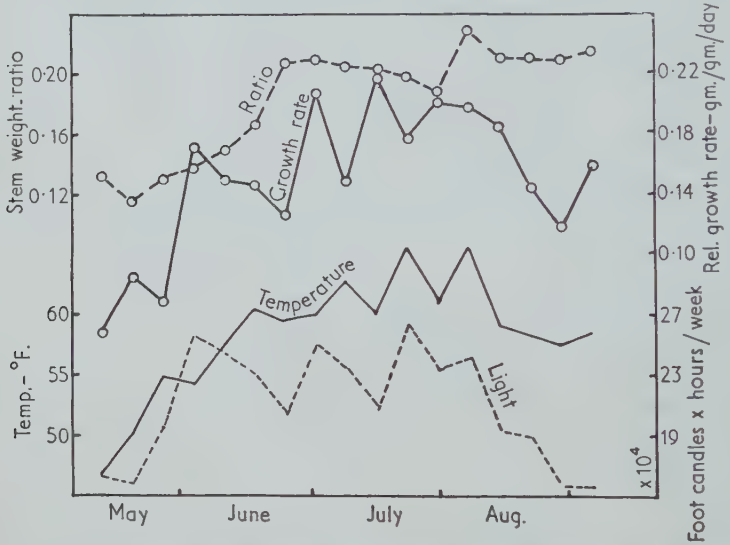


FIG. 10 (1951). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, stem-weight ratio, and the relative growth rate of the stem.

Inspection of the regression (9) confirms that the growth rate, like that for the whole plant (3) is positively linked with both mean temperature and the amount of diurnal light energy.

Relative growth rate of stem (g./g./day)

$$= -0{\cdot}2297 + 0{\cdot}005343T + 0{\cdot}2914L \times 10^{-6} \qquad (9)$$

S.E. $= \pm 0{\cdot}0280$, percentage variation accounted for $= 61$.

In describing the statistical treatment of the data, it was pointed out that the regressions for the stem-weight ratio could not be bulked for the 1950 and 1951 series. In consequence both regressions are given:

1950 series

Final stem-weight ratio (g./g.)

$$= \mathrm{SWR}_F = -0{\cdot}1666 + 0{\cdot}007907T - 0{\cdot}5253L \times 10^{-6} \qquad (10)$$

S.E. $= \pm 0{\cdot}0187$, percentage variation accounted for $= 75$.

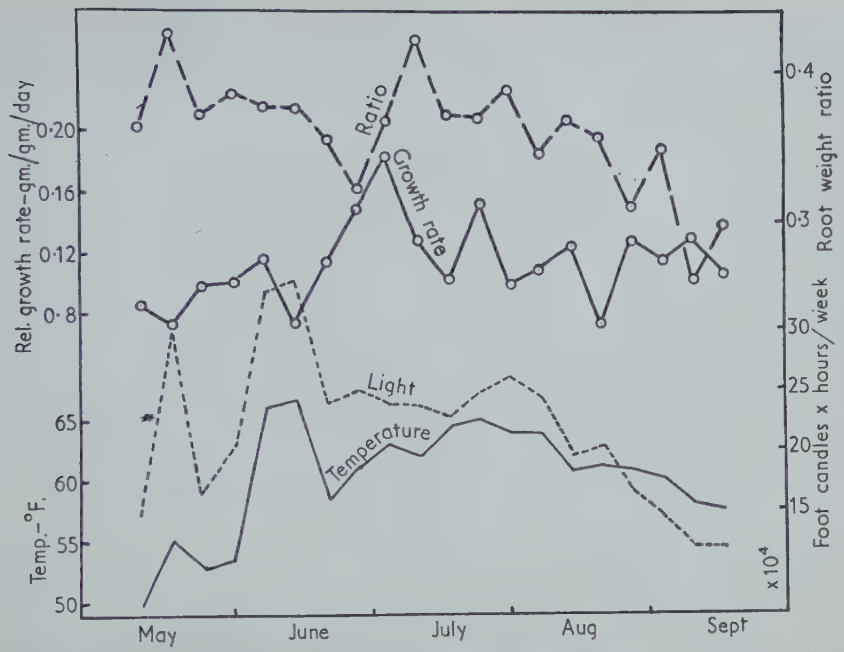


FIG. 11 (1950). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, root-weight ratio, and the relative growth rate of the root.

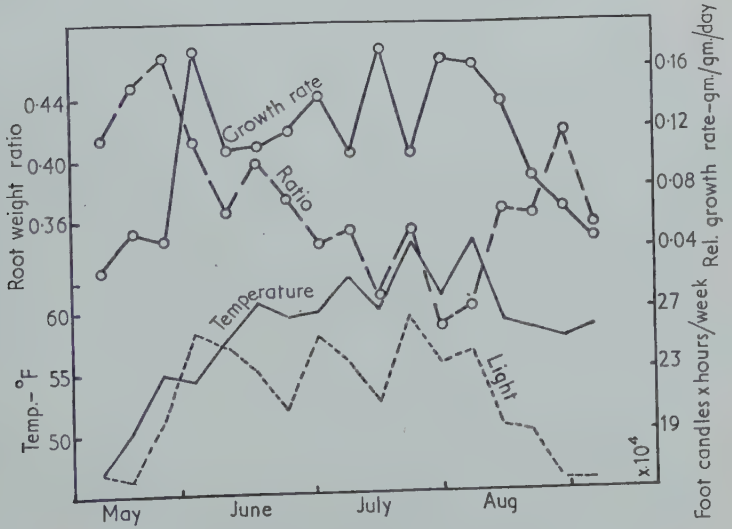


FIG. 12 (1951). Seasonal changes over successive weekly periods in the diurnal mean temperature, daylight, root-weight ratio, and the relative growth rate of the root.

1951 series

$$SWR_F = -0{\cdot}2372 + 0{\cdot}005320T - 0{\cdot}1919L \times 10^{-6} + 1{\cdot}1427\ SWR_I \qquad (11)$$

S.E. = 0·0161, percentage variation accounted for = 91.

Both regressions demonstrate that the stem ratio is positively increased by rising temperature and decreasing light radiation. The difference between the regressions relates to the term for the initial value of the ratio, only in the 1951 series does this term reach significance. Under these conditions, the change in the ratio between the beginning and end of the individual experiments can be almost completely defined by the three factors in the regression.

Root growth. The changes in the relative growth rate and the root-weight ratio are given in Figs. 11 and 12. For the growth rate the fluctuations to some extent follow those for the growth of the whole plant, but the mid-season peak is less well defined. Taking the results of the two years together there is no clear-cut seasonal trend in the ratio: in 1950 the ratio tends to fall slowly between May and September but in 1951 after July the ratio rises again. Regressions (12) and (13) indicate that neither the growth rate nor the ratio are sensitive to changes in the light energy received but that there is some linkage with temperature, positive for the growth rate but negative for the ratio. It should, however, be noted that only a small percentage of the total variance is accounted for in each regression.

$$\text{Relative growth rate of roots (g./g./day)} = -0{\cdot}0974+0{\cdot}003492T \qquad (12)$$

$$\text{S.E.} = \pm 0{\cdot}0341, \text{ percentage variation accounted for} = 19.$$

$$\text{Final root-weight ratio (g./g.)} = 0{\cdot}5768-0{\cdot}003610T \qquad (13)$$

$$\text{S.E.} = \pm 0{\cdot}0422, \text{ percentage variation accounted for} = 14.$$

Discussion

On the basis of the present investigation it is possible to appraise more critically than hitherto the ways in which seasonal changes in light and temperature may operate in controlling the vegetative development of a single species, not only in relation to the growth of the whole plant but also from the viewpoint of the component parts. It has been established that the relative growth rate is positively linked with both temperature and the diurnal light energy received (equation (3)) and that in turn the fluctuations in the growth rate can be interpreted in terms of the effects of these two environmental factors on the net assimilation rate and either the leaf-area or leaf-weight ratios. On the criterion of leaf-area, light is the major factor which determines the level of the net assimilation rate (equation (1)) while the magnitude of the leaf-area ratio is correlated negatively with light intensity but positively with temperature (equation (4)). Alternatively, when leaf weight is substituted for leaf area the net assimilation rate is governed by both light and temperature (equation (2)) but the leaf-weight ratio is independent of either factor.

That the net assimilation rate on an area basis is controlled only by the light factor and on a weight basis by both light and temperature can be further elucidated by considering the effects on the relative growth rates of leaf area and leaf weight in conjunction with the changes induced in the ratio of leaf area to leaf weight. Thus, although the growth rates of both leaf area and

leaf weight are positively dependent only on the light energy received (equations (6) and (7)) these growth rates cannot be simply equated since the ratio of area to weight is negatively linked with the light level and positively related to temperature (equation (5)). Extending this type of analysis to the relative growth rate the influence of the light intensity is compounded of a positive effect on the net assimilation rate and a negative effect on leaf-area ratio while the primary effect of temperature is on the leaf-area ratio, or since the leaf weight is unaffected by both factors on the ratio of leaf area to leaf weight.

Turning to the growth of the stem and roots, the relative growth rate of the stem is increased as both the light level and temperature are raised (equation (9)), but for the roots only temperature is important (equation (12)). This contrast between the two parts also holds for the ratios since while the stem-weight ratio is positively and negatively correlated with temperature and light respectively (equations (10) and 11)) the root ratio is depressed by rising temperature (equation (13)).

It is evident that the weight which can be attached to these conclusions varies considerably since the proportion of the variance accounted for in the individual equations ranges from 91 per cent. (equation (11)) to 14 per cent. (equation (13)). The question therefore arises as to how far such differences indicate either that other factors are operating or that the errors of the experimental procedure are dependent on the type of observation.

That the net assimilation rate (area basis) of *H. annuus* is depressed by a reduction in the level of light has been conclusively demonstrated by previous shading experiments (Blackman and Wilson, 1951*a* and *b*). It has also been demonstrated that exposure in an initial period to a fourfold variation in intensity (0·25–1·0 daylight) has no appreciable residual effect on the subsequent rate of assimilation of *H. annuus* (Blackman and Wilson, 1954). It therefore follows that in the present investigation little error should have been introduced by the seasonal changes in the light radiation received by the plants prior to the individual period of observation. Thus, the fact that 68 per cent. of the variance can be accounted for in terms of the light factor is in line with the previous evidence.

It is somewhat unexpected that statistical analysis has failed to reveal any significant effects of either the mean temperature or the diurnal range of temperature on the net assimilation rate based on leaf area. For barley, Gregory (1926) concluded that this rate was correlated positively with the day temperature and negatively with the night temperature. In addition, one of us (Black, 1955), extending the present study to the seasonal factors controlling the growth of *Trifolium subterraneum* in southern Australia, has also demonstrated that for the net assimilation rate there are positive effects of light radiation and mean maximum temperature during the day and a negative effect of minimum night temperature. Since the same experimental procedure was followed for both *T. subterraneum* and *H. annuus* it is concluded that there are marked specific differences in the temperature reactions. Some support for this view is forthcoming from the investigations of Watson (1947), who

found that while the net assimilation rate of wheat was significantly correlated with the mean diurnal temperature and that of sugar-beet with the daily range, the rate for potatoes was independent of temperature. It must, however, be admitted that no significant effect of total radiation was established for any of these crops and that the variance accounted for in the individual regressions ranged from 6 to 47 per cent.

Turning to a consideration of the net assimilation rate based on leaf weight the only other results are those of Goodall (1945) for the tomato, and it will be recalled that these experiments refer to the environmental conditions of a greenhouse. Nevertheless it is of interest that there is again evidence of a specific difference in relation to the temperature factor. While for *H. annuus* both light and temperature are concerned in determining the rate of assimilation, for the tomato only the influence of the light factor was significant.

Although in the statistical analysis of the assimilation data the seasonal

TABLE I

The Effects of Varying the Light Intensity in an Initial Period on the Subsequent Net Assimilation Rate in Full Daylight

Light intensity in initial period.	Net assimilation rate in full daylight	
	Leaf-weight basis g./g./day.	Leaf-area basis g./dm.²/day.
Expt. 32		
1·0 daylight	0·20	0·077
0·5 „	0·24	0·080
0·25 „	0·26	0·076
Expt. 33		
1·0 daylight	0·31	0·120
0·5 „	0·38	0·119
0·25 „	0·41	0·117

trend, as exemplified by week number was not significant, nevertheless it is possible that some degree of error arose from a varying residual influence of the light level prior to the initiation of the individual weekly experiments. This conclusion is based on a further examination of the data relating to the effects of transference from one light level to another (Blackman and Wilson, 1954). For the two experiments which are most comparable the residual effects of the initial level, on the subsequent rate of assimilation in full daylight, are shown in Table 1. It is evident that on the basis of leaf weight, but not of leaf area, an initial intensity of 0·5 daylight, and more particularly of 0·25 daylight, brings about in the second period a higher rate of assimilation. Since, however, in 1950 and 1951 the maximum difference in the light energy received between consecutive weeks was only 28 per cent. the errors due to changes in light radiation cannot have been large but such residual effects may account for the greater error variance in equation (2), as against equation (1).

In the study of the adaptive changes which follow an alteration in the light level (Blackman and Wilson, 1954) it has already been established that the leaf-area ratio is inversely correlated with light intensity, and that in the period

of 4 to 8 days after transference the order and sign of the change in the ratio is dependent on the magnitude of the ratio at the time of transference, which in turn is related to the light intensity in the pre-treatment period. Thus, in terms of seasonal trends the rise in the light radiation between May and the end of June should lead to a progressive reduction from week to week of the initial and final ratios. Conversely from July to September with a falling light level there should be a corresponding rise in the ratios. Turning to the temperature trends the gain in temperature between May and July should be matched with increases in the ratios followed by decreases during August and September. When the trends for both light and temperature are superimposed it is clear that a complex pattern is produced and that the errors due to the 'seasonal' effect will not have been completely eliminated by considering the changes between the initial and final ratio.

Since the stem-weight ratio and the ratio of leaf area to leaf weight are also positively correlated with temperature and negatively with light, similar considerations apply to the seasonal pattern of the superimposed effects of the two factors. However, on the basis of the previous investigation (Blackman and Wilson, 1954) the residual effects induced by variations in the initial intensity are less for these two ratios than for the leaf-area ratio. Such differences may therefore in part account for the greater precision of equations (5), (10), and (11) over that of equation (4).

That the leaf-weight ratio is apparently so little affected by either the light or temperature factor calls for some comment. In the previous investigation (Blackman and Wilson, 1954), varying the light level either before or after transference brought about no well-defined effects on the proportionate growth of the leaves. Moreover, the results of other shading experiments, as yet unpublished, have shown that relative to plants of *H. annuus* receiving full daylight a reduction in the intensity to quarter daylight at any time between May and September caused on an average of nine experiments less than a 10 per cent. change in the leaf-weight ratio. Very similar results were obtained for *Fagopyrum esculentum* but for other species, such as *Hordeum vulgare* and *Geum urbanum* an equivalent degree of shading caused marked increases in the ratio. Thus it would seem that the magnitude of the changes induced by varying light and temperature is a characteristic of the species.

In the study of the adaptive changes it has been found that a reduction in the light level brought about a marked depression in the proportionate growth of the roots while the residual influence of the level prior to transference was also considerable. Such a residual influence may have contributed to the high error variance of equation (13) but nevertheless it is somewhat surprising that there was no significant effect of light.

It has already been pointed out that the growth rates of the component parts must inevitably be linked with the growth rate of the whole plant and that in consequence the factors which determine the total growth rate will also in part determine the growth rates of the components, even though for the partition of the substrates into stem, leaf, and roots, light and temperature

may operate in a different pattern. Thus, for the growth rates of the stem and the whole plant both light and temperature have a positive influence (equations (3) and (9)), the tendency for decreasing light to increase the proportion of stem (equations (10) and (11)) being more than counterbalanced by the actual gain in weight of the stem as the light radiation is increased. Similarly, though for the root-weight ratio there is a small and negative correlation with temperature, for the relative growth rate the overall effect of temperature is weakly positive.

For *Cucumis sativa* Gregory (1928) observed that when temperature was below the optimal the rate of leaf surface expansion was independent of temperature but proportional to the light intensity in the constant environment rooms. Gregory (1926) over four seasons also sampled on successive occasions a single population of barley plants grown in the open and by measuring departures from a generalized curve of leaf area against time concluded that the rate of change in area was positively related to day temperature and negatively correlated with both the night temperature and the daily radiation as measured with a Callender recorder. With such a procedure it is difficult to allow for ontogenetic drifts or residual influences of a change in the environment, but nevertheless of the two species the reaction of *C. sativa* is more akin to that of *H. annuus* since in this instance too light is the only significant factor determining the relative growth rates of leaf area and weight (equations (7) and (6)). It is to be noted that the error variance is higher for leaf area and this may well be due to the interacting influences of a changing level of light. Thus a rise in the level by increasing leaf weight thereby increases leaf area but, on the other hand, the ratio of area to weight is simultaneously depressed.

From the foregoing considerations it is evident that in some of the less precise regressions the apparent high error variance arises rather from the complexity of the reactions to the light and temperature factors than from the errors of experimental procedure. On the other hand, against the background of the previous evidence (Blackman and Wilson, 1954) the anomalous results for root growth may be due to variations in physiological status at the time of the initial sampling. It is, however, difficult to envisage how the procedure of investigations of this type could be improved without special facilities. If a small environmental room had been available at the time the batches of pots could have been raised under standard conditions, but although the plants might have been of a more comparable physiological status at the beginning of each experiment errors would have been introduced owing to the adaptive changes taking place following on transference from a uniform environment to an environment changing with the season. From this aspect there is much to be said for the present procedure since, on average, the environment to which the plants are subjected before the weekly experimental period will not be very different and therefore the errors due to the residual effects will be small.

When the present regressions are reviewed as a whole the trends are in general consistent and taken together illustrate with some precision the

extent to which seasonal changes in light and temperature may control the vegetative growth and development of a single species. It has, however, been repeatedly emphasized (Blackman and Wilson, 1951*a* and *b*, 1954) that for a further understanding of the basic principles involved such field data cannot stand alone, but nevertheless the results provide a critical basis for investigating either specific problems under more controlled conditions or for planning research on the physiological processes responsible for the observed growth reactions. In this connexion Mitchell (1954) has questioned whether the concept of net assimilation rate is an adequate 'tool for understanding the energy relationships of growth physiology' but this criticism is to some extent not relevant, since the approach of growth analysis is essentially a first step in assessing by relatively simple means the plastic responses to different ecological and environmental conditions. The next phase may well embrace the reinvestigation of some aspects in a series of controlled environments and the results of Mitchell (1953, 1954) for two types of *Lolium perenne* are of interest. As in the present field experiments the relative growth rate was positively linked with temperature and light while the dry weight per unit leaf area was increased by either reducing the temperature or raising the light intensity. Similarly, light more than temperature governed the net assimilation rate but the changes induced in the ratio of leaf weight to the combined weights of stem and roots were more akin to those for leaf-area ratio than for leaf-weight ratio in the sunflower, since there was a positive effect of temperature and a negative effect of light. It would seem therefore that under very different conditions the reactions in some respects were alike for both species but for others dissimilar.

Experiments in controlled environments should be regarded as complementary and no substitute for accurate field or pot culture studies since errors of interpretation are liable to be introduced by translating results from one set of conditions to another. Moreover, except on a very small scale it is both technically difficult and costly to design a controlled environment room where it is possible to attain and maintain a combination of a light intensity approaching that of midsummer daylight with day temperatures equivalent to those of a cool temperate climate.

Although studies in the field and investigations employing controlled environments may supplement each other there remain the still open questions of the physiological pathways through which environmental factors operate. For the light factor the complexity of the reactions and the need for more fundamental knowledge has been discussed in other papers (Blackman and Wilson, 1954; Blackman and Robertson-Cuninghame, 1954, 1955*a* and *b*). The same need also holds for further studies of the reactions of species to seasonal changes in the environment. It will be recalled (p. 543) that for *H. annuus* in England and for *T. subterraneum* in southern Australia the interrelationships between net assimilation rate, light, and temperature are markedly different and it will be shown elsewhere (Black, 1955) that similar disparities also hold for relative growth rate.

Literature Cited

Ballard, L. A. T., and Petrie, A. H. K., 1936: Physiological Ontogeny in Plants and its Relation to Nutrition. I. The Effect of Nitrogen Supply on the Growth of the Plant and its Parts. Aust. Journ. Exp. Biol. Med. Sci., xiv. 135.

Black, J. N., 1955: Light and Temperature and Growth Rate of Subterranean Cloves. Aust. Journ. Biol. Sci., viii. 3. (In the Press.)

Blackman, G. E., Black, J. N., and Martin, R. P., 1953: Physiological and Ecological Studies in the Analysis of Plant Environment. VIII. An Inexpensive Integrating Recorder for the Measurement of Daylight. Ann. Bot., n.s., xvii. 529.

——, and Robertson-Cuninghame, R. C., 1954: Interactions in the Physiological Effects of Growth Substances on Plant Development. Journ. Exp. Bot., v. 184.

——, and ——, 1955*a*: Interrelationships between Light Intensity, Temperature and the Physiological Effects of 2:4-Dichlorophenoxyacetic Acid on the Growth of *Lemna minor*. Ibid., vi. 156.

——, and ——, 1955*b*: Interrelationships between Light Intensity and the Physiological Effects of 2:4-Dichlorophenoxyacetic Acid on the Growth of *Helianthus annuus*. Ibid., vi. 177.

——, and Wilson, G. L., 1951*a*: Physiological and Ecological Studies in the Analysis of Plant Environment. VI. The Constancy for Different Species of a Logarithmic Relationship between Net Assimilation Rate and Light Intensity and its Ecological Significance. Ann. Bot., n.s. xv. 63.

——, and ——, 1951*b*: VII. An Analysis of the Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf Area Ratio and Relative Growth Rate of Different Species. Ibid., xv. 373.

——, and ——, 1954: IX. Adaptive Changes in the Vegetative Growth and Development of *Helianthus annuus* induced by an Alteration in Light Level. Ibid., xviii. 71.

Crowther, F., 1934: Studies in Growth Analysis of the Cotton Plant under Irrigation in the Sudan. I. The Effects of Different Combinations of Nitrogen Applications and Water Supply. Ibid., xlviii. 877.

Fisher, R. A., 1921: Some Remarks on the Methods formulated in a Recent Article on 'The Quantitative Analysis of Plant Growth'. Ann. Appl. Biol., vii. 367.

Goodall, D. N., 1945: The Distribution of Weight Changes in the Young Tomato Plant. I. Dry Weight Changes of the Various Organs. Ann. Bot., n.s., ix. 101.

Gregory, F. C., 1926: The Effect of Climatic Conditions on the Growth of Barley. Ibid., xl. 1.

——, 1928: Studies in the Energy Relations of Plants. II. The Effect of Temperature on Increase in Area of Leaf Surface and in Dry Weight of *Cucumis sativa*. Ibid., xlii.

Mitchell, K. J., 1953: Influence of Light and Temperature on Growth of Ryegrass (*Lolium* spp.). I. Pattern of Vegetative Development. Physiol. Plant., vi. 21.

——, 1954: III. Pattern and Rate of Tissue Formation. Ibid., vii. 51.

Watson, D. J., 1947: Comparative Physiological Studies on the Growth of Field Crops. I. Variations in Net Assimilation Rate and Leaf Area between Species and Varieties and within and between Years. Ann. Bot., n.s., xi. 41.

——, and Baptiste, E. C. D., 1938: A Comparative Physiological Study of Sugarbeet and Mangold with respect to Growth and Sugar Accumulation. I. Growth Analysis of the Crop in the Field. Ibid., n.s., ii. 437.

Williams, R. F., 1936: Physiological Ontogeny in Plants and its Relation to Nutrition. II. The Effect of Phosphorus Supply on the Growth of the Plant and its Parts. Aust. Journ. Exp. Biol. Med. Sci., xiv. 167.

——, 1937: Drift of Net Assimilation Rate in Plants. Nature, cxl. 1099.

——, 1939: Physiological Ontogeny in Plants and its Relation to Nutrition. VI. Analysis of the Unit Leaf Rate. Aust. Journ. Exp., Biol. Med. Sci., xvii. 123.

——, 1946: The Physiology of Plant Growth, with Special Reference to the Concept of Net Assimilation Rate. Ann. Bot., n.s., x. 41.

Breeding Systems in New Zealand Plants

I. *Fuchsia*

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With three Figures in the Text

ABSTRACT

The three New Zealand *Fuchsia* species have heteromorphic flowers previously described as heterostyled. *F. excorticata* and *F. perscandens* are shown to be gynodioecious and *F. procumbens* trioecious. The frequencies of the two forms in several populations of *F. excorticata* are given.

I. INTRODUCTION

GYNODIOECY, or the occurrence of hermaphrodite and female plants in the same species, is an outbreeding mechanism of peculiar interest. On the one hand, the population can have a reliable seed source if hermaphrodite plants are self-fertilized, while, on the other hand, it may derive advantage from the offspring of the obligately cross-pollinated females. There are difficulties, however, in the maintenance of such a system in nature. The hazards of cross-pollination may lead to reduced seed setting on female plants, and thus to a reduced contribution of the genes of the female to the next generation. But more important still is the fact that, whereas in a dioecious species the two sexes contribute approximately equal amounts of chromatin to the next generation, in a gynodioecious species a hermaphrodite plant contributes approximately three times as much chromatin as a female. Genes causing male-sterility are thus in danger of decreasing in frequency, and if females are to survive in a population, some compensating mechanism must exist. Lewis (1941) has shown that where male-sterility is due to a dominant or recessive gene, the females cannot exist in a wild population unless they are more than twice as fertile as the hermaphrodite on the female side. However, Lewis also showed that with cytoplasmic control of male-sterility only a slight advantage in the females is necessary to maintain their frequency in a population.

According to Lewis (1942), more than 90 per cent. of the gynodioecious species so far recorded are in the Labiateae. This paper records gynodioecy in two of the New Zealand *Fuchsias* and describes trioecy in the third.

Although heteromorphic flowers are not described for these species by Munz (1943) in his monograph, New Zealand botanists have long been aware that different flower types exist. Hitherto the classification accepted has been

[Annals of Botany, N.S., Vol. XIX, No. 76, 1955.]

given by Kirk (1892), who recognized three types of plant differentiated by their flowers, and called these 'long', 'mid', and 'short-styled', using the terms employed by Darwin (1877) for a tristylic species such as *Lythrum salicaria*. Although these positions of the stigma were determined in two species, by the degree of protrusion beyond the anthers, and not by the replacement of anthers at three different levels, Kirk believed the situation to be a true case of heterostyly. This confusion with true heterostyly has been maintained by Laing and Blackwell (1951), who introduce an account of Kirk's scheme by a description of heterostyly in *Primula*.

The purpose of this paper is to correct Kirk's descriptions and to provide information on breeding systems and natural populations.

II. *Fuchsia excorticata*

F. excorticata is a small tree from 10 to 30 ft. in height, and is common throughout New Zealand. The flowers are pendulous with very small petals, and the species is gynodioecious.

Hermaphrodite flowers (Fig. 1). One mature flower was collected from each of 126 hermaphrodite trees on Banks Peninsula, Christchurch. The flower length, from top of ovary to apex of stigma, ranged from 24 to 51 mm. with a mean of $35{\cdot}07 \pm 0{\cdot}55$. The degree to which the style protruded above the upper anther whorl, expressed as a percentage of style-length, ranged from nil to 33 per cent. In larger samples from six trees (number of flowers in parentheses), the corresponding figures were: 12–30 per cent. (67), 3–37 per cent. (73), 4–25 per cent. (25), 5–24 per cent. (40), and 4–33 per cent. (41).

It is clear from Kirk's descriptions that his 'short' and 'mid-styled' flower types are hermaphrodites. The 'short-styled' form is illustrated with the stigma at the same level as the upper whorl of stamens, while the flower illustrated as 'mid-styled' has the style protruding beyond the upper stamens for 30 per cent. of its length (Kirk, 1889). The right-hand pair of hermaphrodite flowers in Fig. 1 show the two extremes of style protrusion in a sample from one tree, and in this respect are almost identical with Kirk's two types. Kirk noted that it would not be difficult to find trees intermediate between these two types, and Cheeseman (1925) while accepting the two forms notes that they apparently grade into one another. I find it impossible to distinguish two distinct forms in the field, and as the 'mid' and 'short-styled' flowers can even be collected from the same tree, it is obvious that the classification is artificial.

Although trees cannot be differentiated by the relative positions of style and anthers, they may differ in flower size. Genetical studies are, of course, not practicable, so that an indirect approach must be made to determine whether this variation has a genetical basis. To minimize possible environmental effects, measurements of the tube (hypanthium) and of style-length were made in two hermaphrodite trees growing with their branches touching (Fig. 1, *a–b*). An analysis of variance (Table I) showed a significant difference between trees for both characters. Variation in stylar protrusion, on the other

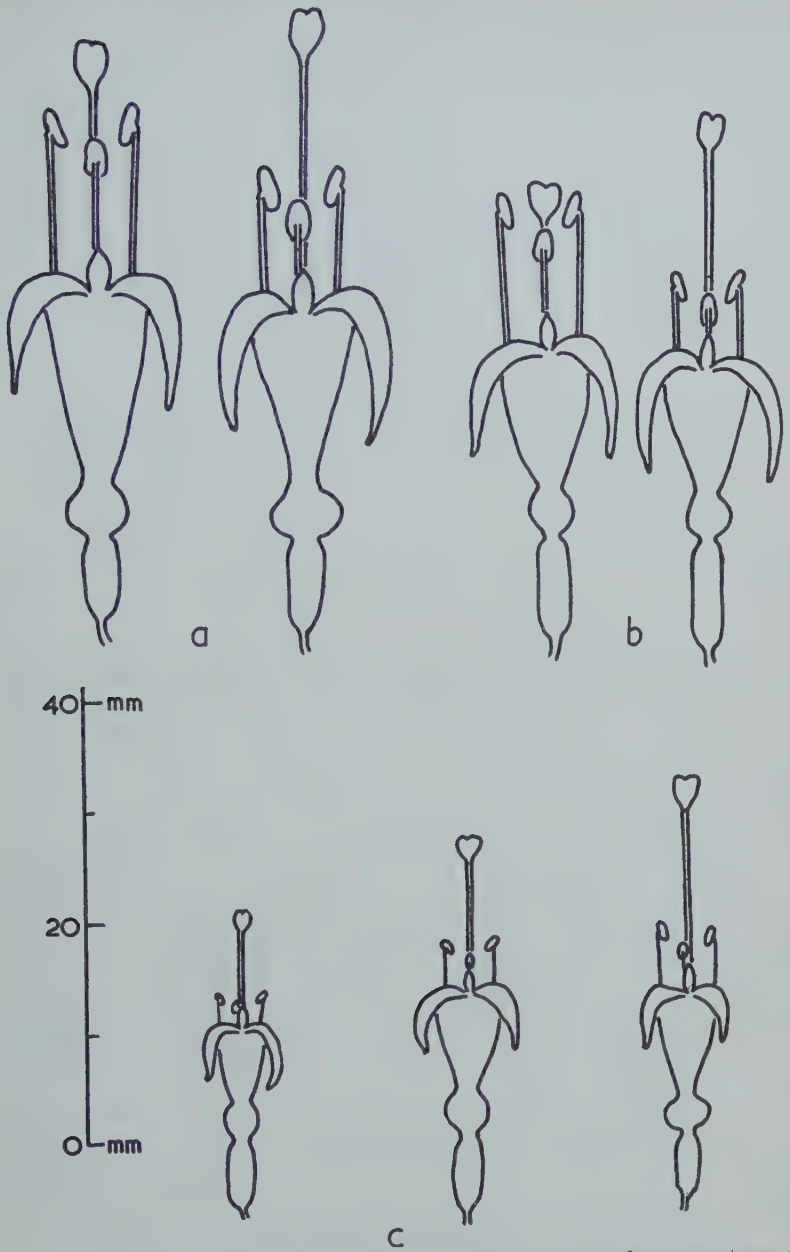

FIG. 1. *Fuchsia excorticata*. *a–b*, pairs of hermaphrodite flowers from two trees growing together, showing differences in style protrusion on the same tree, and differences in size between trees; *c*, female flowers from three trees.

hand, was not significantly different. The correlations between tube and style-length were 0·667 and 0·638 respectively, while those between tube-length and length of second stamen whorl were 0·661 and 0·606. In the original sample

of 126 flowers the corresponding figures were 0·770 and 0·321. These rather loose correlations between the size of the floral parts could cause the variation in degree of style-protrusion observed (cf. Fig. 1*b*).

The observed variation in hermaphrodite flowers may be summed up as follows. Trees may differ in absolute length of style and tube, and this is probably under polygenic control. Within trees the degree of stylar protrusion varies, and this variation is approximately the same for all trees examined.

Female flowers (Fig. 1). A sample of one flower from each of 32 trees on Banks Peninsula gave flower lengths ranging from 19 to 31 mm. with a mean of 26·7±0·05. Thus most female flowers are smaller than hermaphrodites,

TABLE I

Analysis of Variance for Tube and Style Length in Two Neighbouring Trees of F. excorticata

Tube length.	df.	s.s.	m.s.	F.
Between trees	1	643·49	643·49	329·5
Within trees .	138	269·45	1·953	—
Total . .	139	912·94	—	—

Style length.	df.	s.s.	m.s.	F.
Between trees	1	3,649·56	3,649·56	411·3
Within trees .	138	1,224·58	8·874	—
Total .	139	4,874·14	—	—

a point not illustrated by Kirk (1889). The difference in flower size between female trees growing in proximity can be quite obvious, and would be expected if this character is under polygenic control.

As the tiny staminodes project for only about 2 mm. above the tube, the style is very prominent and Kirk described it as 'long-styled', but as shown above it is usually shorter than in hermaphrodite flowers.

Pollination. Pollen was examined from three trees and found normal. This conflicts with observations by Beer (1921), who included *F. excorticata* in a list of species having irregular pollen development or all sterile pollen. It is possible that female flowers were studied.

Fruiting is always prolific, and artificial pollination showed that hermaphrodite flowers are self-fertile. Bagged female flowers set no fruits, and as these females gave hybrids when crossed with *F. procumbens*, the possibility of apomixis is ruled out. Cross-pollination by two nectar-seeking native birds, the tui and bell-bird, has also been recorded as important (Potts, 1870; Myers and Atkinson, 1923), but cross-pollination by wind is probably more difficult, as the pollen is held together by mucilage in masses, and is only blown with difficulty from the dehisced anther.

Natural populations. F. excorticata grows from sea-level to about 3,800 ft., and extends throughout New Zealand, covering 12 degrees of latitude. The populations in Table II are listed from north to south, and cover as much of

the distribution area as was practicable. They extend over 9 degrees of latitude and include three populations from high altitudes, the remainder being from between sea-level and 1,000 ft. Each count was obtained by walking through bush for distances up to a mile and scoring all trees bordering the road.

TABLE II

Frequency of Flower Types in Natural Populations of F. excorticata

Population.	Miles from previous station.	Hermaphrodite.	Female.	Total.	Percentage females.
North Island:					
1. Auckland; Filters Rd. and Mt. Atkinson . .	—	56	38	94	40·42
2. Ruahine Range; Track to Rangiwahia Ski Club Hut (3,000–3,800 ft.) . . .	210	58	26	84	30·95
3. Wellington; Khandallah Reserve .	120	69	16	85	18·82
South Island:					
4. Queen Charlotte Sound; Momorangi Bay . .	30	64	26	90	28·88
5. Nelson; Wairoa Gorge . .	50	55	26	81	32·10
6. Nelson; Maruia Springs Hotel (2,300 ft.) . .	80	56	21	77	27·27
7. Christchurch; Summit Rd., Banks Peninsula . .	110	126	32	158	20·25
8. Taramakau River; below Harper's Pass (2,000–2,500 ft.) . . .	80	151	72	223	32·29
9. Aickens Rly. Station	10	103	42	145	29·00
10. Dunedin; Leith Valley . .	200	105	41	146	28·08
11. Dunedin; Highcliffs, Otago Peninsula . . .	8	79	31	110	28·18
12. Bluff; coastal track .	120	97	4	101	4·12
Total . .	—	1,019	375	1,394	—

The numbers of the two types in samples from twelve districts are given in Table II. In eight of these, from widely separated districts and different altitudes, the frequency is remarkably uniform, ranging from 27·27 to 32·29 per cent. of female plants. An explanation of the frequencies observed must await knowledge of the genetic basis of male-sterility and of the relative 'fitness' of the two types. Genetical experiments would take many years with

this tree, but a short-cut is being explored using artificial hybrids between the different types of *F. procumbens* and *F. excorticata*. Undoubtedly the female type has been present in this species for a considerable time, and the high frequencies observed in all but one sample must indicate a greater 'fitness' of female plants, if male-sterility should prove to be controlled by a single gene. Two of the components of 'fitness' are the number of seeds produced and their percentage germination. Only a rough measure of the first variable will be possible, as each tree may produce some hundreds of many-seeded edible fruits. It may be noted that Kirk considered that the 'long-styled' trees (females) produced the most fruits.

The only counts of gynodioecious populations of which I am aware are the brief records given by Darwin (1877) which show little regularity in the proportions of the two forms. Two populations of *Thymus serpyllum* had 12 and 0 females respectively among hundreds of plants, and three populations of *Nepeta glechoma* contained, respectively, all females, all hermaphrodites, and a preponderance of hermaphrodites. Two small counts of *Echium vulgare* gave 11 females, 4 hermaphrodites, and 14 females, 16 hermaphrodites, 2 intermediates. *Cnicus palustris* and *acaulis*, *Plantago lanceolata*, and *Scabiosa arvensis* are recorded as having a preponderance of hermaphrodites.

III. *Fuchsia perscandens*

This species was described by Cockayne and Allan (1927). It is not common, and is a slender scrambling liane, which can form compact bushes in exposed situations. The flowers are pendulous with tiny petals, and could be conveniently described as smaller copies of the flowers of *F. excorticata*.

Allan (1927) described a flower type which is hermaphrodite. He writes, however, 'I have not seen more than one type (long-styled) of floral structure in *F. perscandens*'. This is not the correct use of the term 'long-styled' in Kirk's sense, as he applied it to plants which are actually females.

I have collected flowers from both hermaphrodite and female plants in the Riccarton Bush, Christchurch, and these are illustrated in Fig. 2. As in *F. excorticata*, the females are smaller than the hermaphrodites.

IV. *Fuchsia colensoi*

The validity of this species requires further investigation. Allan (1927) notes that the description was not based on a type specimen, but on a collection of variable material, and that natural hybrids of *F. excorticata* and *F. perscandens* could conform to the description of *F. colensoi*. He suggests, however, that one or more true shrubby forms may exist.

I have examined a hybrid swarm of *F. excorticata* × *perscandens* in the Riccarton Bush, Christchurch, containing plants which would be referred to *F. colensoi*, and find that both hermaphrodite and female plants exist. The flowers are similar to those of *F. excorticata*.

V. *Fuchsia procumbens*

This is a very slender-stemmed, trailing species, and is confined to the coast in the north of New Zealand where it is not common. The flowers contrast with the two previous species in being erect, apetalous, and having the two whorls of anthers at almost the same level. After examining herbarium specimens and eleven populations, it was found that this species is trioecious. The forms described have retained their differential characters for three seasons in a glass-house.

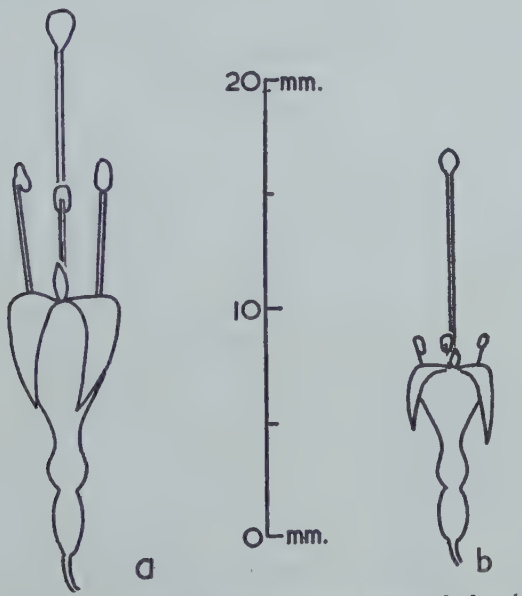

Fig. 2. *Fuchsia perscandens*. *a*, hermaphrodite flower; *b*, female flower.

Hermaphrodite (Fig. 3). The characteristic flowers of such plants have a large globose stigma about 2 mm. in diameter and level with the anthers. Intermixed with these one may find flowers with aborted stigmas, and in these aberrants the styles vary in length, from those of normal height to those which reach only to the mouth of the tube. Hermaphrodite flowers are self-fertile, and by the end of a season these plants are covered with fruits. The imperfect flowers set no fruits.

This form was known to Cheeseman (1914) and probably to Kirk, as is shown by their remarks on fruit-setting. It was not, however, distinguished from the type A male of this paper, and both forms were called 'mid-styled' (Kirk, 1892).

I have found this in only one population, growing with the type B male.

Male, type A (Fig. 3). This differs from the hermaphrodite in having slightly shorter stamens and a stigma which is only $\frac{3}{4}$ mm. in diameter. The stigma is almost always level with the anthers, and often shrivels when the flowers open.

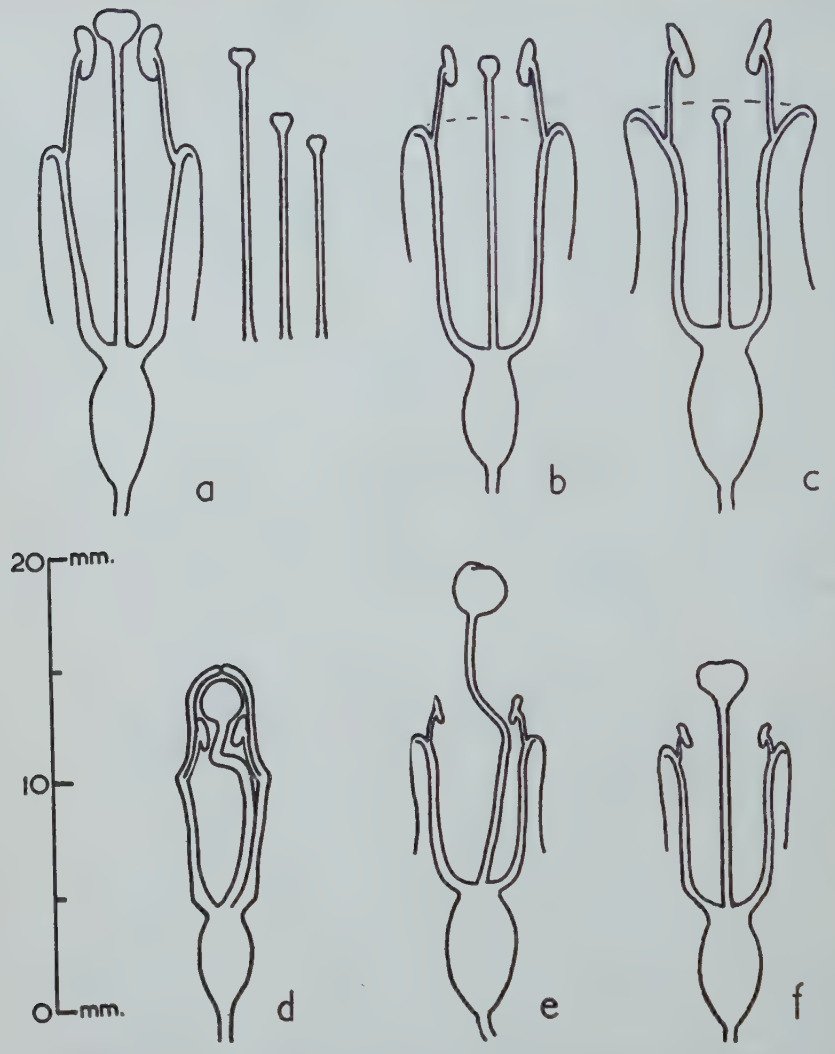

FIG. 3. *Fuchsia procumbens*. *a*, hermaphrodite flower, and styles of male flowers occasionally formed on hermaphrodite plants; *b*, male, type A; *c*, male, type B (*F. kirkii*); *d*, section of flower bud of female, type B; *e*, female, type B; *f*, female, type A.

With one exception, mentioned below, these flowers have never set perfect fruits on selfing or crossing. It is possible, however, to obtain parthenocarpic fruits with persistent flowers. The following observations were made:

(*a*) Six mature flowers with shrunken stigmas on self-pollination gave three parthenocarpic fruits with persistent flowers.

(*b*) Five flowers, with unshrunken stigmas when bud-pollinated, yielded four parthenocarpic fruits with persistent flowers.

(*c*) Of 84 flowers on an isolated plant, only 1 produced a fruit and this was parthenocarpic.

(*d*) Ten flowers used as females in a cross with a hermaphrodite gave two fruits which were parthenocarpic with persistent flowers.

(*e*) In 1950 an isolated plant in the field at Ngahau produced about 100 flowers, and the one fruit set contained seeds.

Thus the cause of female-sterility is probably due to some characteristic of the stigma. Possibly functional ovules are present, but are rarely fertilized.

Cheeseman's illustration (1914) is of a flower with an unshrivelled stigma.

Two populations were found pure for this type, and in a third it grew with the female, type B.

Male, type B (Fig. 3). The tube and anther length are the same as in type A, but the style is always short, never showing beyond the mouth of the tube. This is Kirk's 'short-styled' form. The stigma has the same size and characteristics as the type A male.

Four selfed flowers set no fruit. Kirk, writing of three localities in which only this form had been recorded, stated: 'At Tryphena Bay Professor Hutton and myself examined hundreds of flowers but saw no trace of fruit; subsequently I had the same experience at Mine Bay and again at Whangaruru.' On a plant at Tryphena which set some 150 flowers I found only one fruit towards the end of the season and this contained seeds. Thus these flowers are like the type A males, in being able to set a rare 'illegitimate' fruit due to some cause as yet unknown.

This was the second flower-type discovered, and was first collected by Kirk in December 1867 'on the beach of Great Barrier Island' (Hooker, 1871). The locality was almost certainly Tryphena. The only form then known was the one described in this paper as type A female, and which was already named *F. procumbens*. Specimens from Great Barrier were sent to Hooker who first considered them merely another sexual form of *F. procumbens*, but finally described it under the new name of *F. kirkii* (Hooker, 1871), and gave an excellent illustration of a flower. Munz (1943) has retained *F. kirkii* as a species. However, Kirk (1892) realized that this form was wrongly classified, and since then New Zealand taxonomists have rightly assigned it to *F. procumbens*. It may be found growing intermixed with the female, and seed from these females gives females and '*F. kirkii*' in approximately equal proportions.

This type was found in five populations. In two it grew alone; in two it was intermixed with the female type A, and in one with the hermaphrodite.

Female, type A (Fig. 3). The flowers are approximately two-thirds the size of the preceding types. Because of the shorter tube and tiny staminodes the style protrudes, and this led Kirk to classify the flower as 'long-styled'. A typical plant had style-lengths ranging from 10 to 13 mm. The stigma is always large and globose, and of the same type as in the hermaphrodite. The flowers set no fruit in isolation but are fertile with pollen from males and hermaphrodites.

This was the first type of flower recorded and is the common form of the female. It has been excellently illustrated by W. J. Hooker (1842). The

illustrations of Kirk (1892) and Cheeseman (1914), on the other hand, give no indication of the smaller size of the flower or of the large stigma.

Two populations have only this type; in two others it grows intermixed with the type B male.

Female, type B (Fig. 3). This differs from the previous type in having a longer style, which, on the one plant available, ranged from 12 to 17 mm. in length. The style becomes bent when confined within the small female flower-bud, and the distortion persists in mature flowers. These styles are almost as long as those of the hermaphrodite. I have found this variant in only one population, growing with the type A male.

VI. Breeding Systems in the Genus

The three New Zealand *Fuchsia* species belong to the *Skinnera* section of the genus. It would be of interest if heteromorphic flowers occur in *F. cyrtantroides* of Tahiti, which is the one other species of this section. The type specimen is hermaphrodite (Moore, 1942). Miss Marie C. Neale, botanist at the Bishop Museum, Honolulu, has kindly examined for me the four sheets of this species in their herbarium (1315, 1341, 17005, and a collection by M. L. Grant), and all are from hermaphrodite plants. Considering, however, the small number of specimens seen, it is possible that female plants may yet be found in this species.

This isolated Pacific section of the genus is probably not unique among the *Fuchsias* in having gynodioecious species. Within the *Encliandra* section, which is distributed from Mexico to Panama, Munz (1943) describes the flowers of seven species as hermaphrodite, four species as 'perfect or pistillate', one species as 'perfect or imperfect', one as 'dioecious or polygamous', and three as dioecious.

When the genetical basis of the differences in flower type in *F. procumbens* is known, it may provide information as to the way dioecy has evolved in the genus, and also whether or not gynodioecy has played a part in the process.

Summary

1. *F. excorticata* and *F. perscandens* are gynodioecious, and female flowers are smaller than hermaphrodites.

2. Differences in flower size between hermaphrodite plants and also between females in *F. excorticata* are probably under polygenic control.

3. Of twelve widely separated populations of *F. excorticata*, eight showed female percentages between 27·27 and 32·29, the remaining percentages being 40·42, 20·25, 18·82, and 4·12.

4. *F. procumbens* is trioecious, and two style lengths have been found in males and females. The type B male has previously been described as *F. kirkii*.

5. Gynodioecy probably occurs also in the *Encliandra* section of the genus.

Acknowledgements

This work was begun while on the staff of the Botany Department, Auckland University College. I am grateful to Professor V. J. Chapman for facilities given, and to the Research Grants Committee for a grant for field work.

I am grateful to several colleagues for aid in sampling populations, and in particular to Miss L. B. Moore, Botany Division, Department of Scientific and Industrial Research. Dr. J. B. Hair, also of Botany Division, kindly checked the paper, and Mr. E. J. Beuzenberg supplied the illustrations.

Literature Cited

Allan, H. H., 1927: Illustrations of Wild Hybrids in the New Zealand Flora. V. Genetica, ix. 499–515.

Beer, R., 1921: Notes on the Cytology and Genetics of the Genus *Fuchsia*. J. Genet., xi. 213–27.

Cheeseman, T. F., 1914: Illustrations of the New Zealand Flora. Wellington.

—— 1925: Manual of the New Zealand Flora (2nd ed.). Wellington.

Cockayne, L., and Allan, H. H., 1927: Notes on New Zealand Floristic Botany including Descriptions of New Species, &c. (No. 5). Trans. and Proc. Roy. Soc. N.Z., lvii. 48–72.

Darwin, C., 1877: The Different Forms of Flowers on Plants of the Same Species. London.

Hooker, W. J., 1842: Icones Plantarum, t. 421.

Hooker, J. D., 1871: Icones Plantarum, xi, plate 1083.

Kirk, T., 1889: The Forest Flora of New Zealand. Wellington.

—— 1892: On Heterostyled Trimorphic Flowers in New Zealand Fuchsias. Trans. N.Z. Inst., xxv. 261–8.

Laing, R. M., and Blackwell, E. W., 1951: Plants of New Zealand. 5th ed., revised. Whitcombe & Tombs Ltd., New Zealand.

Lewis, D., 1941: Male Sterility in Natural Populations of Hermaphrodite Plants. New Phyt., xl. 56–63.

—— 1942: The Evolution of Sex in Flowering Plants. Biol. Rev., xvii. 46–67.

Moore, J. W., 1942: New Species of Dicotyledonous Spermatophytes from Tahiti. Occ. Papers, Bishop Museum, xvi. 1–24.

Munz, P. A., 1943: A Revision of the Genus *Fuchsia* (Onagraceae). Proc. Calif. Acad. Sci., xx. 1–138.

Myers, J. G., and Atkinson, E., 1923: The Relation of Birds to Agriculture in New Zealand. N.Z. Journ. Agr., xxvi. 299–306.

Potts, T. H., 1870: On the Birds of New Zealand. Trans. N.Z. Inst., iii. 59–109.

Laccase and Tyrosinase in some Wood-rotting Fungi

BY

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Abstract

Several species of wood-rotting fungi have been examined for phenol oxidases. *Polystictus sanguineus*, a white rot, produces laccase, secreting it into the medium. This enzyme has been studied and partly purified. *Phellinus cryptarum*, another white rot, shows varied production of phenol oxidase according to the medium used for growth. Laccase only is produced on malt medium, whereas laccase and tyrosinase result after growth on a glucose and salt medium. *Lentinus lepideus*, a brown rot, produces tyrosinase on several media and this enzyme appears to be entirely intracellular.

Introduction

EVIDENCE regarding phenol oxidases in wood-rotting fungi indicates a marked difference between the species, but few have been studied in any detail. That most species of white rots differ from brown rots in the type of phenol oxidase produced, now seems certain. White rots commonly secrete a laccase into the surrounding medium (Fähreus, 1952; Law, 1950; Lindeberg and Fähreus, 1952), whereas brown rots do not. Thus they may be differentiated fairly certainly by such tests as that of Bavendamm (applied extensively by Davidson *et al.* (1938)) and of Preston and McLennan (1948). Certain brown rots, on the other hand, form tyrosinase (Law, 1950) which does not react with the Bavendamm substrates and which appears to occur intracellularly. Some species may form both enzymes, as in the case of certain Hymenomycetes investigated by Lindeberg and Holm (1952), and others appear to produce no phenol oxidases at all (Fähreus, 1949).[1]

Several more species have been examined to try to gain further information regarding the types and distribution of the phenol oxidases. Preliminary experiments are described concerning the occurrence of laccase and tyrosinase in certain species which will be used for further investigation.

Experimental Procedure and Results

Growth media

Several media were used for the growth of the species examined.

1. 1 per cent. Difco Malt Solution.

[1] A further study of the enzymic oxidation of lignin by van Vliet (Biochim. biophys. Acta, xv, 2, 1954) has appeared since this paper has been in the press.

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]

2. *Medium G*
   Glucose 30 gm.
   Marmite 2 gm.
   KH_2PO_4 1·0 gm.
   Distilled water to 1,000 ml.

3. *Medium F*
   As in medium G, but in place of marmite the following were added:
   Ammonium sulphate 1 gm.
   $CuSO_4$ 0·1 mg.
   Aneurin 5 mg.

The various species were grown on 100 ml. of medium in 250 ml. conical flasks or in Roux bottles at 25° C.

Substrates

Phenol oxidases act on a number of substrates (Law, 1950) and a few of the most useful ones for differentiation of laccase and tyrosinase have been selected. The scheme used for detection and investigation of the enzymes is very similar to that tabulated by Lindeberg and Holm (1952).

Enzyme.	Guaiacum.	Guaiacum + Catechol.	Catechol.	Hydroquinone.	p-cresol.	Tyrosine.	Gallic acid.	Inhibition by CO.
Laccase	+	..	+	+	+ milky	−	+	−
Tyrosinase	−	+	+	−	+ brown	+	−	+

Guaiacum and tyrosine were used qualitatively only, while the other substrates were used also in the manometric experiments. Both mycelium and medium were tested from time to time qualitatively at pH 4–5, and at their own final pH, and active samples selected for further investigation.

Manometric procedure

Oxygen uptake was measured using the following conditions: 0·5 ml. of the substrate solution containing 5 mg. of catechol, hydroquinone, or p-cresol (or the equivalent of other substrates—final concentration 0·011 M.) in the side-arm; 0·2 ml. 10 per cent. KOH was usually added to the centre well, though in fact CO_2 is not produced in the reaction; 0·5 ml. of citric acid–phosphate buffer (McIlvaine), enzyme solution and water were added to the centre. The total volume was 4·2 ml. The flasks were equilibrated with air and experiments carried out at 25° C. In the carbon monoxide experiments, mixtures of 80 per cent. CO and 20 per cent. O_2 were used, air being the control. These experiments were carried out in the dark.

White rots

Polystictus versicolor (Linn.) Fr. was used for comparison in some of the tests since much of the information regarding laccase has been obtained by a study of this species. (Fähreus, 1952; Dion, 1952; Law, 1950.)

Polystictus sanguineus (L.) Mey. Bose and Sarkar (1937) reported the presence of laccase in this species. On all of the media employed in these experiments it produced a very active extracellular laccase which reacted in the same way as that of *P. versicolor*. The activity appeared to be greatest at 18–21 days. This laccase was partly purified and more fully investigated.

Phellinus cryptarum. (Karst.) Growth of this species on malt and on G medium was good but not satisfactory on F medium. The phenol oxidase activity varied markedly according to the medium. After growth on malt, weak laccase activity was shown in the medium, while G medium produced weak laccase and strong tyrosinase, both of which appeared to be confined to the mycelium. The tests were applied after 21–25 days' incubation. Manometric tests were carried out to confirm the presence of these enzymes.

Brown rots

Coniophora cerebella. (Pers.) Law (1950) reported negative reactions for this species and stated that, if present, the phenol oxidase was not in an active form. Further investigation has shown that on both F and G media, and also when grown on malt agar, the mycelium gives a weak reaction with guaiacum in the presence of catechol, indicating the presence of a tyrosinase. Several batches were grown and tested and there was great variation in the optimum time for development of the enzyme. Some reacted after 12 days, losing their activity after longer periods; some developed the enzyme later and reacted at 38 days of growth. The enzyme was always weak and seldom appeared active enough to test quantitatively. One sample showed oxygen uptake with catechol as substrate. The medium from some of the flasks gave a very weak guaiacum test (without catechol) after standing for a few hours.

Lentinus lepideus. Fr. Malt, and F and G media were used for the growth of this species and on all a very active tyrosinase was produced. The enzyme appears to be entirely intracellular, since no tyrosinase reaction was obtained in the media. The mycelium reacted with the tyrosinase substrates tabulated above and the activity varied with age. Usually the enzyme was easily detectable at about 21 days' growth, but was found to be at its optimum at about 40 days. This species grew very slowly and the enzyme in one case was still active at 70 days growth. The action of the tyrosinase was further investigated.

Lenzites trabea. (Pers.) Fr. No phenol oxidase has been detected in this species though there was good growth on all the media.

Laccase from Polystictus sanguineus

P. sanguineus was found to produce laccase when grown on malt, G, or F medium. The enzyme is extracellular and the media in every case gave

reactions with the various phenolic substrates, and was found to be quite similar in this respect to those given by *P. versicolor* grown on malt medium. Fig. 1 shows the uptake of oxygen when samples of media from the two species acted on the substrates. The optimum pH (Fig. 2) of the laccase from *P. sanguineus* on malt medium was 4·4 using catechol as substrate. With hydroquinone the enzyme showed a lower optimum (3·6–3·8). The range of pH and the optimum will almost certainly vary with different substrates but since activity was high with both these substrates at pH 4·5, buffer of this pH was used throughout the testing.

The enzymes produced by *P. sanguineus* when grown on G and on F media were compared. Growth was normal on G medium and at 18–21 days the medium showed high phenol oxidase activity. On F medium growth was less

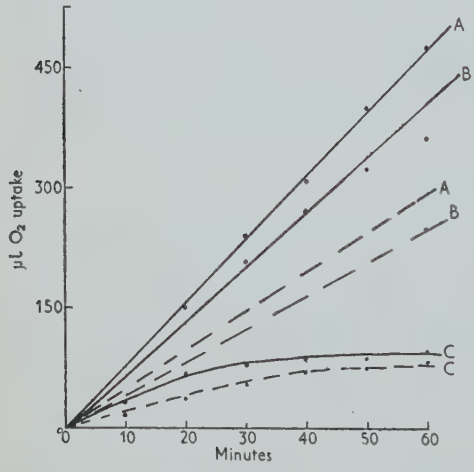

FIG. 1

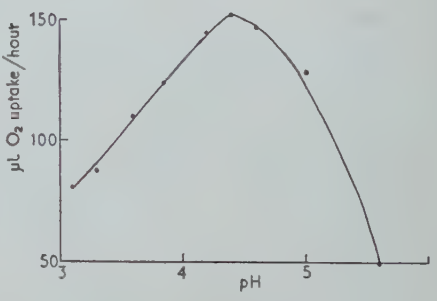

FIG. 2

FIG. 1. Uptake of O_2 by *P. sanguineus* (unbroken line) and *P. versicolor* (broken line) with hydroquinone (A), catechol (B), and p-cresol (C), using 3 ml. malt medium. Controls using original medium + substrates—nil.

FIG. 2. pH range of laccase from *P. sanguineus* using 1 ml. of medium (malt) with catechol as substrate.

vigorous and rather erratic, though often an enzyme of high activity was obtained. On this medium *P. sanguineus* produced a brown soluble pigment which was easily dialysed away in distilled water. Both media produced laccase which was apparently similar to that found in the malt medium.

As one of the criteria of a laccase as distinct from tyrosinase is the fact that laccase activity is not affected by CO, these media were tested as described in an atmosphere of 20 per cent. O_2 and 80 per cent. CO. Figs. 3 and 4 show the action of the enzyme produced on G and F media respectively and the action of CO on the media. Before testing, the media were dialysed for 36–48 hours against distilled water. It can be seen that under these conditions there is no effect of CO on the enzyme and therefore the phenol oxidase in the medium is laccase. If tyrosinase were present CO should inhibit the action with catechol and p-cresol but not with hyroquinone, since tyrosinase has no

action on this substance. Both tyrosinase and laccase have been found to occur together in *Polystictus hirsutus* (Higuchi, 1953) and in *Phellinus cryptarum* (this communication) and since tyrosinase might be present as an intracellular enzyme, the mycelium was tested. For this purpose a homogenate was used. There was considerable laccase activity shown, but no inhibition with CO. Mycelia obtained from 3 up to 6 weeks' growth were tested but no indication of the presence of tyrosinase was shown.

The uptake of oxygen with gallic acid as substrate was measured with several samples of media, a typical result being shown in Fig. 10.

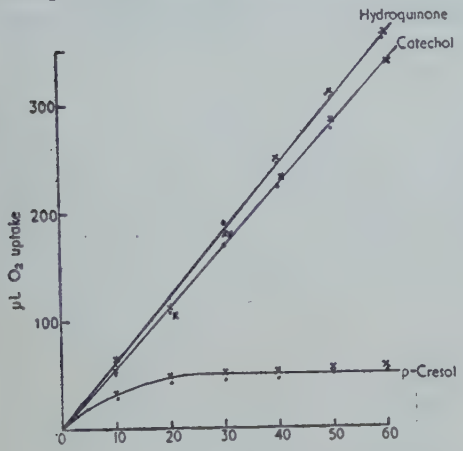

FIG. 3

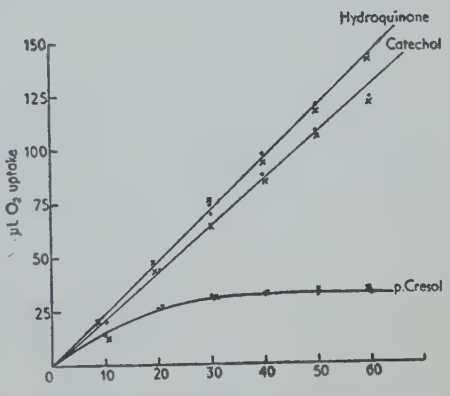

FIG. 4

FIG. 3. Effect of CO on laccase produced on G medium, using 1 ml. of dialysed medium—dry weight = 0·32 mg./ml. (. = air, x = CO.)

FIG. 4. Effect of CO on laccase produced on F medium, using 1 ml. of dialysed medium—dry weight = 0·13 mg./ml. (. = air, x = CO.)

PURIFICATION OF THE LACCASE OF *P. SANGUINEUS*

Purifications were carried out to try to increase the activity and to find whether or not there was any change in the comparative rates of reaction with the three substrates. Change of substrate activities has been shown with potato tyrosinase in various stages of purification and considered to be indicative of the presence of more than one enzyme or active centre.

Twenty bottles each containing 100 ml. of G medium were inoculated and the fungus allowed to grow for 18 days at 25° C. The medium was poured off and filtered, and the mycelium ground and extracted several times with water, the extractions being added to the medium. The total volume was 3,300 ml. (stage I). A small quantity of this was dialysed. The bulk was precipitated with ammonium sulphate at approximately 0·8 saturation and allowed to stand overnight. The precipitate was collected, dissolved in water, and the volume made to 800 ml. The insoluble material was centrifuged off, and discarded. The solution was precipitated a second time and again allowed to stand overnight. The precipitate was collected, dissolved in 250 ml. of water, and dialysed against running tap water overnight (stage II). This solution was slightly

coloured. The colour was removed by the addition of 10–15 ml. of Ca gel suspension (20 mg./ml.) at pH 7, stirring and immediately centrifuging. There was considerable loss of enzyme but the resulting solution was quite clear and colourless. The pH was then adjusted to 4–4·5 with dilute acetic acid, and 50 ml. of Ca gel added, stirred, and allowed to stand for 15 minutes

TABLE

Medium.	Stage.	Volume.	Dry wt. mg./ml.	μl O_2/hour/ml. Catechol.	Hydro-quinone.	p-cresol.	QO_2 Catechol.	Hydro-quinone.	p-cresol.
G	I	3,300	5·5	104	116	34	19	21	6
	II	290	0·7	419	537	71	599	767	101
	III	90	0·02	68	76	16	3,400	3,800	800
	I dialysed		0·2	95	94	33	475	470	165
F	I dialysed	800	0·13	124	145	33	954	1,115	254
	II	85	0·09	84	93	22	933	1,030	244
	III	20	0·045	124	153	24	2,755	3,400	533

and then centrifuged. Most of the enzyme, as indicated by the guaiacum test, was adsorbed. The solution was treated again with a further 10 ml. of gel. The two lots of gel were combined, washed twice in 250 ml. of M./300 citric–phosphate buffer at pH 4·4, centrifuging after each washing. The enzyme

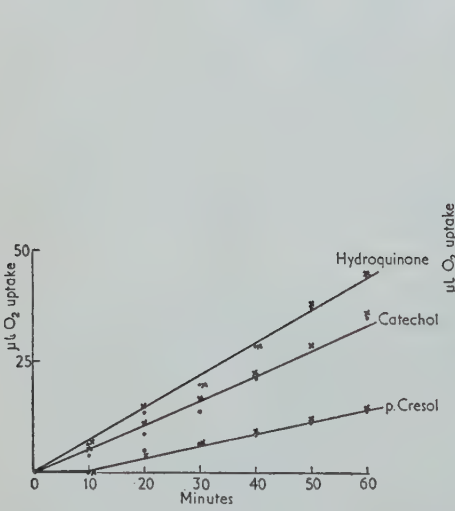

FIG. 5

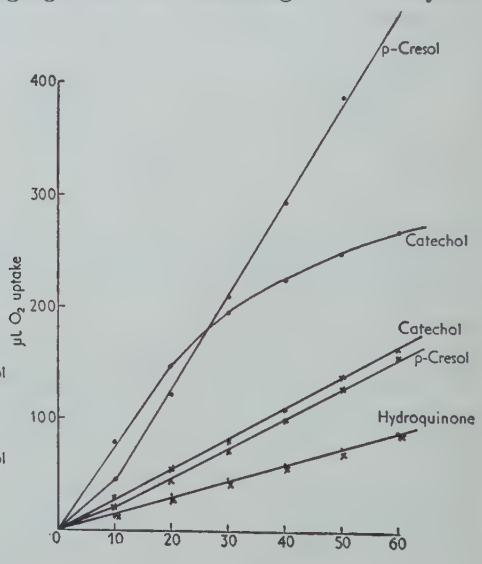

FIG. 6

FIG. 5. Action of the enzyme from *Phellinus cryptarum* on malt medium using 3 ml. of undialysed medium. (. = air, x = CO.)

FIG. 6. Action of a homogenate of *Phellinus cryptarum* grown on G medium using 3 ml. of homogenate—dry weight = 21 mg./ml. (. = air, x = CO.)

was eluted with 0·2M Na_2HPO_4 pH 8·3, using 35 ml. for the first elution followed by 15 ml. for two subsequent elutions, each being allowed to stand for 15 minutes with occasional stirring. The elutions were combined and dialysed against distilled water for 60 hours with frequent changes of water (stage III).

The stages in the purification of the enzyme have so far been followed only

by estimating the activity per dry weight of the solution. The results of one preparation are given in the Table. The solutions at all stages maintained their activity for at least several days if kept in the cold, and often for more than a week. Freeze drying was found to decrease the activity greatly.

A preparation from F medium is also described in the Table. This medium was dialysed and then precipitated and treated according to the method described above. In this case precipitation with ammonium sulphate achieves little, since the dialysed medium has so little solid matter present. Such a medium, consisting entirely of dialysable substances has great advantages for purposes of purification of the enzymes produced.

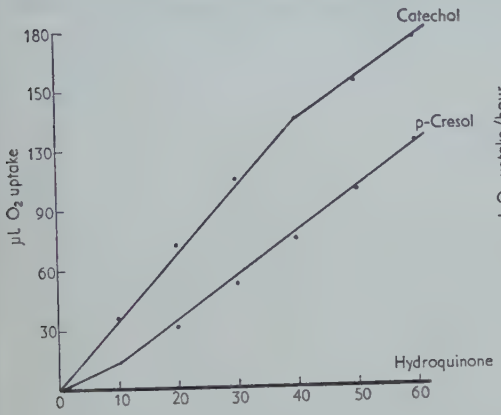

Fig. 7

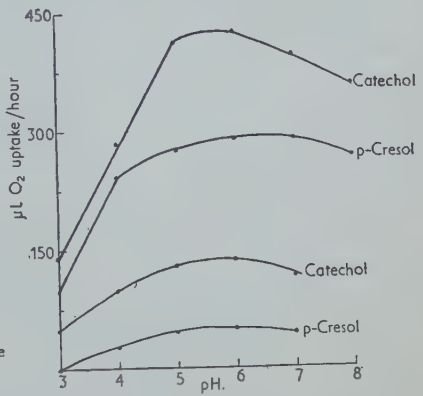

Fig. 8

Fig. 7. Action of a homogenate of *Lentinus lepideus* on catechol, p-cresol, and hydroquinone at pH 4·6. (Homogenate 1 ml.; dry wt.: 6·4 mg.)

Fig. 8. pH range of the phenol oxidase of *Lentinus lepideus* (F medium). The 2 top curves given by 1 ml. homogenate (dry wt.: 9·3 mg.), the 2 lower by 1 ml. extract (dry wt.: 3·9 mg.).

The figures of both preparations indicate that the laccase produced is extremely active. The final solution obtained from F medium was tested with all substrates in the presence of CO and no inhibition was obtained. The optimum pH was determined, and found to be 4·4 with catechol as substrate, as in the case of the enzyme tested in crude malt medium (Fig. 2). Further investigations of the enzyme from such a medium may serve to show whether this laccase consists of more than one component.

Phellinus cryptarum. This species was grown on malt or on G medium and the mycelium and medium tested for phenol oxidases at pH 4·5. The enzymes varied according to the medium. After a period of about 3 weeks' growth on malt, a weak laccase was found in the medium, a typical reaction being shown in Fig. 5. The mycelium showed a similar reaction and there was no sign of the presence of tyrosinase. The laccase activity of the medium in this case decreased considerably on dialysis, and was not restored by the addition of copper. It will be of interest to compare this laccase with that produced by *P. sanguineus*.

The phenol oxidase produced as a result of growth on G medium showed a distinct contrast as indicated in Fig. 6. In this case there was little or no activity

shown in the medium, but a homogenate of the mycelium contained a very active tyrosinase and a weak laccase. The action on catechol and on p-cresol was markedly inhibited by CO whereas the action on hydroquinone was unaffected, indicating a mixture of the two enzymes. This tyrosinase shows a much greater 'cresolase' than 'catecholase' activity in contrast to that of *Lentinus lepideus* described below.

Tyrosinase from *Lentinus lepideus*

When grown on malt or F medium *Lentinus lepideus* produced an active tyrosinase which appeared to be wholly confined to the mycelium. The latter reacted with guaiacum in the presence of catechol giving an intense blue colour, and greatest activity was shown usually at about 40 days' growth. As extraction of the enzyme was very difficult, tests were carried out on homogenates

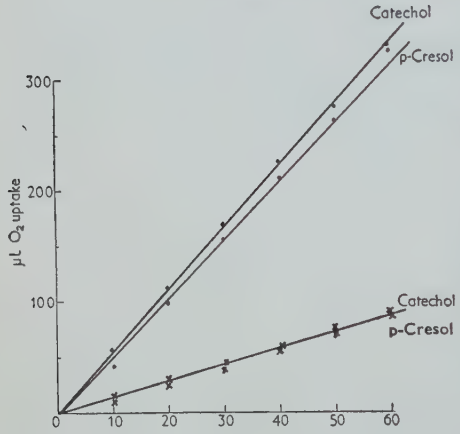

Fig. 9

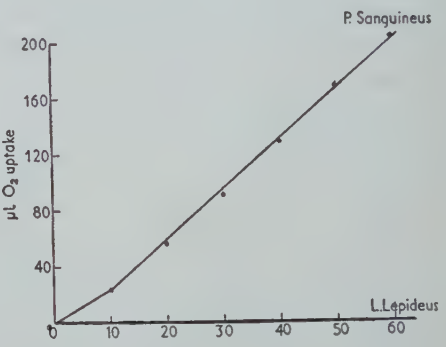

Fig. 10

Fig. 9. Effect of carbon monoxide (20% O_2—80% CO mixture) on the phenol oxidase of *Lentinus lepideus*. Volume of homogenate, 2 ml. = 40 mg. dry wt. (. = air, x = CO.)
Fig. 10. Action of laccase (*P. sanguineus*) and tyrosinase (*L. lepideus*) on gallic acid.

in water. Catechol, p-cresol, and hydroquinone were used as substrates for comparison with the action of the laccase from *P. sanguineus* and the experiments were carried out at pH 4·5–5 (Fig. 7). The optimum pH was determined in the homogenate of mycelium grown on both malt and F media. The pH range of activity of the enzyme is wide and though the optimum with both catechol and p-cresol appears to be pH 6, the slope of the curve is very gradual (Fig. 8). Such a curve may be due possibly to the presence of more than one enzyme. Experiments were not usually carried out at pH above 7 since the substrates then show increasing blanks.

The effect of CO on the activity of the enzyme was very marked with both substrates (Fig. 9). This inhibition and the fact that the enzyme does not act on hydroquinone confirms the presence of a tyrosinase.

Brown rots, in contrast to white, have no action on gallic acid, one of the Bavendamm substrates. In Fig. 10 curves are shown comparing the action of the medium containing the laccase from *P. sanguineus* with that of a homo-

genate of *Lentinus lepideus*. Several homogenates were tested and no action on gallic acid was obtained. It therefore appears that this fungus contains a tyrosinase and, as might be expected, no laccase is present.

Discussion

Several species have been tested according to the scheme given previously, and the three substrates catechol, hydroquinone, and p-cresol, used in air and in 80 per cent. CO–20 per cent. O_2, form a useful method for distinguishing between tyrosinase and laccase. The two former substrates are obviously the best of these for testing laccase activity under the conditions adopted. However, p-cresol, even if not under optimum conditions, is useful for purposes of comparison, and laccase and tyrosinase may be distinguished to some extent by the difference in colour of the end-products.

Polystictus sanguineus, a white rot, produces a phenol oxidase similar to that formed by *P. versicolor*. The enzyme is extracellular, being secreted into the medium, and shows the properties of a laccase regarding its substrate specificity and insusceptibility to carbon monoxide. It has been partly purified and further tests will be done to determine whether there is more than one component. In the case of the laccase of *P. versicolor*, Dion (1952) suspected the presence of more than one enzyme. Under the conditions of growth *P. sanguineus* showed no evidence of tyrosinase production, laccase being the only phenol oxidase produced on the various media employed.

Another white rot, *Phellinus cryptarum*, shows a distinct variation in phenol oxidase production according to the medium used for growth. On malt medium only laccase activity is shown, whereas on G medium, in addition to laccase, a strong tyrosinase is produced. The outstanding character of this tyrosinase is its high 'cresolase' activity and lower 'catecholase' activity compared with that produced by *Lentinus lepideus* grown on F. medium. Since tyrosinases from different sources show varying catecholase and cresolase activities (Nelson and Dawson, 1946) it is likely that different species of wood-rotting fungi will show this variation, and that the activities of the tyrosinase of one species will vary according to the medium.

Lentinus lepideus, a brown rot, produces only tyrosinase which is intracellular and extremely difficult to obtain free from the mycelium. Homogenates of the fungus oxidized the typical tyrosinase substrates having no action on either hydroquinone or gallic acid. The enzyme was markedly inhibited by carbon monoxide. The wide pH range and the absence of a sharp optimum suggests that this tyrosinase contained more than one enzyme or component and further comparison with the tyrosinase of *Phellinus cryptarum* may serve to elucidate this point.

Two other brown rots were tested, *Coniophora cerebella* giving weak tyrosinase tests, but *Lenzites trabea* giving no phenol oxidase reactions. Fähreus (1952) has shown that, in the case of *P. versicolor*, various conditions affect the production of laccase, and doubtless this applies to all species. In the case of *Coniophora cerebella* considerable variation in the length of time of production

of the enzyme was noticed. It is therefore probable that a number of factors must be considered before conclusions can be drawn regarding phenol oxidase production.

The function of phenol oxidase is as yet unknown, though the production of laccase by white rots has been associated with their ability to decompose lignin. Dion (1952) showed some slight oxidation of lignin with the enzyme from *P. versicolor*. On the other hand, Higuchi (1953) found no evidence of oxidation with *P. hirsutus*. It is possible that the reaction is too slow to be measured manometrically, or that other factors are involved. Nord and Vitucchi (1948) has suggested that the decomposition of lignin may in some way be linked with that of cellulose. The natural substrates and the actual function of these enzymes have yet to be elucidated.

Summary

1. Three species of wood-rotting fungi showing positive reactions for phenol oxidases have been studied in further detail.

2. *Polystictus sanguineus* produces a laccase similar to that of *P. versicolor*. The enzyme has been partly purified. *Phellinus cryptarum*, another white rot, shows a variation of phenol oxidase according to the medium.

3. *Lentinus lepideus*, a brown rot, produces tyrosinase intracellularly. The enzyme was identified in homogenates of the mycelium.

I wish to express my thanks to Dr. W. P. K. Findlay of the Forest Products Research Laboratory, Princes Risborough, for his helpful advice and for supplying the cultures. My thanks are also due to Mr. H. Tristram of the Botany Department of this College for his advice concerning the mycological aspects of this work.

Literature Cited

Bose, S. R., and Sarkar, S. N. (1937): Enzymes of some Wood-rotting Polypores. Proc. Roy. Soc., B, cxxiii. 193.

Davidson, R. W., Campbell, W. A., and Blaisdell, D. J. (1938): Differentiation of Wood-destroying Fungi by their Reaction on Gallic or Tannic Acid Medium. J. Agric. Research, U.S.A., lvii. 683.

Dion, W. M. (1952): Production and Properties of a Polyphenol Oxidase from the Fungus *Polyporus versicolor*. Can. J. Botany, xxx. 9.

Fähreus, G. (1949): On the Oxidation of Phenolic Compounds by Wood-rotting Fungi. Ann. Agr. Roy. Coll. Sweden, xvi. 618.

—— (1952): Formation of Laccase by *Polyporus versicolor* in Different Culture Media. Physiol. Plant. v. 284.

Higuchi, T. (1953): Biochemical Study of Wood-rotting Fungi. (1) Studies on the Enzymes which cause Bavendamm's Reaction. J. Japanese Forestry Soc. xxxv, No. 3, 1.

Law, K. (1950): Phenol Oxidases in Some Wood-rotting Fungi. Ann. Bot., n.s., xiv. 69.

Lindeberg, G., and Fähreus, G. (1952): Nature and Formation of Phenol Oxidases in *Polyporus zonatus* and *P. versicolor*. Physiol. Plant., v. 277.

—— and Holm, G. (1952): Occurrence of Tyrosinase and Laccase in Fruit Bodies and Mycelia of some Hymenomycetes. Ibid. 100.

Nelson, J. M. and Dawson, C. R. (1946): Tyrosinase. Advances in Enzymology, iv. 99.

Nord, F. F., and Vitucchi, J. C. (1948): Certain Aspects of the Microbiological Degradation of Cellulose. Advances in Enzymology, viii. 253.

Preston, A., and McLennan, E. I. (1948): The Use of Dyes in Media for distinguishing between Brown and White Wood-rotting Fungi in Culture. Ann. Bot., n.s., xii. 53.

Iron, Manganese, Ash, and Nitrogen in some Plants from Salt Marsh and Shingle Habitats

BY

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Abstract

Analyses for iron, manganese, ash, and nitrogen are recorded for some plants from salt marshes and shingle hooks. Ash is much higher, and iron and manganese are much lower, than in plants from underwater lake muds, woodland soils of varying humus content and acidity, and semi-aquatic marsh, fen, and bog soils. Since both lake muds and salt-marsh soils are deficient in oxygen, with iron and manganese presumably mobile as divalent ions, the difference in concentration of the two elements in plants from the two types of habitat requires explanation. It is suggested that the much greater total ion concentration of the salt-marsh soil solution may depress iron and manganese assimilation through ionic antagonism.

Introduction

In an earlier paper (Mayer and Gorham, 1951) submerged freshwater plants were shown to accumulate much larger quantities of iron and manganese than most plants in semi-aquatic and woodland habitats. It was suggested that the anaerobic and strongly reducing conditions in the lake muds were responsible for these high plant levels of iron and manganese, by maintaining the two elements in their reduced and more soluble divalent state. Since salt-marsh soils may also be largely anaerobic, and salt-marsh plants are known to have a high mineral content, it seemed worth while to investigate the amounts of iron and manganese in such species. Therefore, in the latter part of August 1952, twelve samples of six characteristic species were collected for this purpose on the younger salt marshes at Blakeney Point in Norfolk. For comparison, three species were collected from shingle hooks in the same locality. The earlier plant samples were analysed for total ash and nitrogen as well as the metals; these determinations were also made on the present series.

The Area

The general ecology of Blakeney Point is discussed in detail by Tansley (1939), who has summarized the extensive studies of Oliver and his collaborators (cf. also Oliver, 1913; Oliver and Salisbury, 1913). Salisbury (1922) has analysed the surface soils of the area for pH, ignition loss, and calcium carbonate. The shingle soils are circumneutral, those of the salt marshes are slightly alkaline. The organic contents (as ignition loss) of both groups

[Annals of Botany, N.S. Vol. XIX, No. 76, 1955.]

average between 5 and 10 per cent. dry weight. Their levels of calcium carbonate are usually very low, the averages for the general areas examined in this study being 0·1 per cent. dry weight for the shingle soils and 2·6 per cent. for those of the salt marshes.

The profile morphology of Blakeney Point salt-marsh soils has also been investigated; in conjunction with measurements of redox potential, tests for ferrous and ferric iron, and for sulphides (E. Gorham, in preparation). These studies indicate that the extremely impervious muds of the main marshes are largely depleted of oxygen within the rooting depths of the vegetation. Iron and manganese must be rather freely mobile in such soils, to allow the extensive accumulations of black ferrous sulphide observed where organic decay has brought about extremely reducing conditions; or to account for the rusty precipitations of ferric hydroxide where air or aerated water has been introduced into the predominantly anaerobic soil. Such oxidizing conditions are chiefly encountered along well-drained creek margins, around long decayed root channels or worm tracks, and in more permeable sand layers which are often found a short distance beneath the muds. The sandier soils of the marsh margins are also well aerated.

Methods

Whole-plant tops were sampled from typical habitats during August 1952 and were immediately water-washed very carefully. Their glossy surfaces made cleaning relatively easy and efficient. The material was then dried in an improvised oven at 70–90° C. Before analysis the plants were again dried, at 105–110° C.

Ash. The total mineral matter was determined by ignition at about 700° C., since a lower temperature did not dispose of all the carbon.

Nitrogen. This constituent was estimated by the usual micro-Kjeldahl method, on duplicate samples.

Iron. The dry plant material was digested with a mixture of nitric, perchloric, and sulphuric acids (Sandell, 1944, p. 278) and diluted to volume. Iron was then estimated on an aliquot by the thiocyanate method in 50 per cent. acetone, hydrogen peroxide being added as oxidizing agent and ethylene glycol monobutyl ether for colour stability (Snell and Snell, 1949, vol. 2, p. 310). A photocolorimeter with blue filter was used for the colour comparison. Duplicate samples were tested.

Manganese. Another aliquot digested as above was boiled with added sulphuric and phosphoric acids and potassium periodate (Sandell, 1944, p. 314), after which colour was read with a green filter. Again duplicate samples were tested.

Results

The analyses for both shingle and salt-marsh plants are given in Table I. Iron and manganese are expressed as milligrammes per hundred grammes dry weight, ash, and nitrogen as per cent. dry weight.

TABLE I

Iron, Manganese, Ash, and Nitrogen in Shingle and Salt-marsh Plants

Species.	Iron	Manganese	Fe/Mn	Ash	Nitrogen	Site descriptions.
	mg./100 g. dry weight.		ratio.	% dry weight.		
Honckenya peploides	4	2	2	17	1·0	Sandy shingle, well aerated.
Silene maritima	6	7	1	13	2·9	Sandy shingle, well aerated.
Limonium binervosum	22	2	11	9	2·0	Sandy shingle, well aerated.
Limonium vulgare	20	4	5	20	2·0	*Aster–Salicornia* marsh; mud largely anaerobic.
Limonium humile	19	2	10	15	2·3	*Aster–Salicornia* marsh; mud largely anaerobic.
Aster tripolium (leaves)	7	4	2	38	1·9	*Salicornia–Pelvetia* marsh; mud largely anaerobic.
Aster tripolium (leaves)	7	2	4	42	1·4	*Aster–Salicornia* marsh; mud largely anaerobic.
Aster tripolium (stems with flower buds)	2	2	1	25	0·6	
Pelvetia canaliculata	8	6	1	19	1·2	*Salicornia–Pelvetia* marsh, free-floating in shallow pan.
Salicornia perennis	5	2	3	38	1·5	Margin of marsh, with *Halimione portulacoides* and *Suaeda fruticosa*; muddy sand well aerated.
Salicornia stricta	9	4	2	39	1·8	Edge of creek, with *Halimione* and *Suaeda maritima*; sand well aerated.
Salicornia stricta	5	1	5	44	1·6	Well-drained high-level marsh, with *Halimione* and *Suaeda maritima*; sandy mud well aerated.
Salicornia stricta	6	2	3	44	2·6	Edge of shingle bank, few plants; thin mud layer well aerated.
Salicornia stricta	7	1	7	41	1·7	*Salicornia–Pelvetia* marsh; mud largely anaerobic, inside edge of pan.
Salicornia stricta	9	4	2	45	1·3	*Salicornia–Pelvetia* marsh; mud largely anaerobic.
Salicornia stricta	5	6	1	42	1·6	*Aster–Salicornia* marsh; mud largely anaerobic.

Iron. The values for this constituent range from 2 to 22 mg./100 g. dry wt. The lowest amount is that in the *Aster* flowering stems. The three *Limonium* species, including one from shingle and two from salt marshes, average more than twice the iron contents of the other plants. There seems to be little difference in iron level between plants of aerobic and anaerobic soils.

Manganese. The maximum concentration of this element is 7 mg./100 g. dry wt., in *Silene maritima.* Two samples of *Salicornia stricta* contain as little as 1 mg./100 g. dry wt. Again, there appears little difference in plants of oxidizing or largely reducing soils.

Ash. Total mineral matter is generally higher in the salt marsh species than in those of the shingle, with *Salicornia* and *Aster* containing about 40 per cent. of ash. Within the two types of habitat the genus *Limonium* provides the lowest figures. There is little variation in ash content of *Salicornia stricta,* whether on aerobic or anaerobic soils.

Nitrogen. As might be expected, the flowering stems of *Aster* exhibit the smallest nitrogen concentration, 0·6 per cent. dry wt. The highest value, 2·9 per cent., comes from *Silene maritima.* Plants of shingle and salt marsh, or aerobic and largely anaerobic salt marsh, apparently differ little in this respect. *Salicornia stricta* shows considerable variability in nitrogen level, ranging from 1·3 to 2·6 per cent. dry weight.

Discussion

The main interest of the present results lies in a comparison with data from other types of habitat. In view of the extreme variation and skewed distribution of iron and manganese concentrations in the plants examined earlier (Mayer and Gorham, 1951), such a comparison is probably best made from median values for each habitat type. Table II provides such a series of very

Table II

Nitrogen, Ash, Iron, and Manganese (approximate median values) in Plants from Various Natural Habitats

Habitat.	Soil pH.	Nitrogen	Ash	Iron	Manganese	Fe/Mn.
		% dry weight.		mg. per 100 g. dry weight.		
Salt marsh	>7	1·7	41	7	3	2·6
Lake mud	5·6–7·1	3·7	18	106	76	1·0
Marsh and fen	4·6–7·3	2·2	6	25	35	0·7
Fen and bog	2·9–4·5	1·8	4	19	55	0·3
Woodland.	4·6–7·6	2·3	9	26	23	1·1
Woodland.	2·5–4·5	2·1	6	24	44	0·6

approximate medians, obtained by fitting cumulative frequency distributions for element concentrations roughly by eye, and intersecting at the 50 per cent. level. While these medians are only rather crude approximations, it is believed that the differences between habitat types are of sufficient magnitude to make serious errors in the order of values unlikely.

It appears from Table II that salt-marsh plants contain much less iron and manganese than plants from other habitats, especially those growing on reducing lake muds, which are high in both elements. This is true even on other than dry weight comparisons. For instance, if iron and manganese are calculated per unit of total mineral ash, or per unit of total nitrogen, the salt-marsh plants are still very low. (The underwater plants also remain highest in iron; but because of low ash and nitrogen contents the plants of acid fens and bogs become highest in manganese on these bases.) A major problem

TABLE III

The Acidity of Freshly Ground Tops from Plants of Shingle, Salt Marshes, and Freshwater Lakes

Species.	Distilled water added, % of fresh tissue weight.	pH glass electrode.
Lake plants:		
Nitella	100	6·36
Sparganium minimum	100	6·28
Potamogeton alpinus	100	6·28
P. praelongus	100	6·16
Elodea canadensis	100	6·10
Littorella uniflora	100	5·04
Nuphar lutea	200	4·92
Isoetes lacustris	100	4·44
Salt marsh plants:		
Pelvetia canaliculata	50	7·96
Suaeda maritima	0	6·47
Limonium humile	50	6·00
L. vulgare	50	5·96
Aster tripolium	0	5·91
Salicornia stricta (lower marsh)	0	5·90
S. stricta (upper marsh)	0	5·84
Shingle plants:		
Honckenya peploides	25	5·86
Senecio jacobea	25	5·54

here is why salt-marsh plants, with a high mineral uptake and growing on reducing muds where iron and manganese must be rather mobile, should be so low in these elements, especially when plants on similarly reducing lake muds are so high.

It was considered that the acidity of plant sap might differ in the two habitats, thus affecting the movement of iron and manganese within the plant tissues. An attempt was made to assess this factor by grinding fresh tops, usually with the addition of some distilled water, and measuring the pH of the product. The results, given in Table III, suggest that this factor is unlikely to be decisive, although the crudity of the technique does not allow certainty on this point.

It also seemed possible that air might diffuse more easily into the periodic-

ally exposed salt-marsh soils than into the permanently waterlogged lake muds, so that iron and manganese would be precipitated before reaching the root surfaces of the salt-marsh plants. However, while rusty precipitations may be observed around their roots, these are far from universal. Very many roots show little or no trace of ferric hydroxide, while others exhibit rusty sheaths over widely varying proportions of their length. Sometimes, it is true, new roots grow along old rusty root channels, where the ferrous sulphide deposited during intense decomposition has later been oxidized, upon the entry of air once the organic matter has mostly decayed away. None the less, the surface soils of the main salt marshes are in general highly impervious, and probably remain anaerobic to within a few millimetres of the top, even during the longer periods of exposure. In connexion with aeration, some of the analysed species from both lake muds and salt marshes possess well-developed root aerenchyma or air spaces.

A factor which may well be of great importance is the far higher total ion concentration of the salt-marsh soil solution. Ferrous and manganous ions will be of far less importance in the salt marsh than in the much more dilute soil solution of the lake muds, i.e. they will form a much smaller fraction of the total ions presented to the root surfaces. Thus these elements should account for a much smaller proportion of the total mineral uptake of the salt-marsh species. And in fact, as a percentage of ash, the median iron content of underwater lake plants is more than 25 times that of salt marsh species, while the median manganese value is more than 50 times as great.

The importance of ion antagonism for plant growth and nutrient uptake has been stressed by Olsen (1942, 1950, 1953). There seems little reason to doubt that it will have a profound influence upon the accumulation of iron and manganese.

The ratio of iron to manganese is also a matter of interest, since it varies widely in different habitats. The median values in Table II decline with increasing acidity of the soil. In the case of plants on woodland and semi-aquatic soils in the Lake District, this is clearly due to a distinct increase of manganese in the more acid sites, iron remaining much the same. Moreover, Fe/Mn ratios are lower in plants of semi-aquatic soils than in comparable woodland sites—again largely because manganese is higher. It is interesting that in spite of great divergence in absolute levels, the Fe/Mn ratios of plants on circum-neutral lake muds and slightly alkaline salt-marsh soils follow the general trend in relation to soil pH.

To turn to total mineral content, the salt-marsh species are remarkable in yielding up to 45 per cent. of ash. The maximum value recorded for the other habitats was 21 per cent. in *Littorella* growing on lake mud. This high level of mineral matter in the salt-marsh plants is responsible for making them appear the lowest in nitrogen on the dry weight basis. If nitrogen is recalculated per unit of combustible organic material, it then becomes slightly higher in the salt-marsh species than in those from woodland and semi-aquatic soils. However, on this basis the submerged lake plants contain almost

twice as much nitrogen as the other plants, which may of course reflect a lower proportion of carbonaceous supporting tissues in the former.

Acknowledgements

We should like to thank Dr. M. Y. Stant, Miss B. Stephen, and Mr. Than Tun for advice and technical assistance.

Literature Cited

Mayer, A. M., and Gorham, E., 1951: The Iron and Manganese Content of Plants present in the Natural Vegetation of the English Lake District. Ann. Bot., n.s., xv. 247–63.

Oliver, F. W., 1913: Some Remarks on Blakeney Point, Norfolk. Journ. Ecol., i. 4–15.

—— and Salisbury, E. J., 1913: Topography and Vegetation of Blakeney Point, Norfolk. Trans. Norfolk Norw. Nat. Soc., ix.

Olsen, C., 1942: Water Culture Experiments with Higher Green Plants in Nutrient Solutions having Different Concentrations of Calcium. Compt. Rend. Trav. Lab. Carlsberg, xxiv. 69–97.

—— 1950: The Significance of Concentration for the Rate of Ion Absorption by Higher Plants in Water Culture. Ibid., xxvii. 291–306.

—— 1953: The Significance of Concentration for the Rate of Ion Absorption by Higher Plants in Water Culture. IV. The Influence of Hydrogen Ion Concentration. Physiol. Plant., vi. 848–58.

Salisbury, E. J., 1922: The Soils of Blakeney Point: A Study of Soil Reaction and Succession in Relation to the Plant Covering. Ann. Bot., xxxvi. 391–431.

Sandell, E. B., 1944: Colorimetric Determination of Traces of Metals, 1st edition. New York.

Snell, F. D., and Snell, C. T., 1949: Colorimetric Methods of Analysis, vol. 2, 3rd edition. New York.

Tansley, A. G., 1939: The British Islands and their Vegetation. Cambridge University Press.

THE JOURNAL OF

ECOLOGY

Edited for *The British Ecological Society*

By H. GODWIN

Vol. XLIII, No. 2. July 1955

The Journal of Ecology is published twice a year. Subscription price is 50*s.* net per volume, post free. Single copies cost 35*s.* net, postage extra. Index to volumes 1–20, £1.

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